

## Quality of Peanut (*Arachis hypogaeae* L.) Kefir with Variation in Ragi Starter Concentration and Long Fermentation

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### ABSTRACT

One of the fermented milk products, namely kefir, is increasingly popular because it has many health benefits. Peanut juice has a high enough protein content that it can be used as a substitute for animal milk. The purpose of this study was to determine the quality of peanut kefir with variations in the concentration of ragi tape inoculum and fermentation time. This research method used 3 variations in the concentration of tape yeast (1, 2, and 4%) and long fermentation time (24, 48, and 72 hours). The results showed that the highest total LAB was in 4% ragi tape inoculum with a fermentation time of 24 hours ( $4.4 \times 10^8$  cells/mL) and the lowest was in 4% tape yeast inoculum with a fermentation time of 72 hours ( $9.8 \times 10^7$  cells/mL) and total yeast between  $1 \times 10^4$  cells/mL -  $3 \times 10^5$  cells/mL) and alcohol produced <1%. Total acid obtained between 6% - 17.6%. The increase in total acid is proportional to the decrease in pH. The pH of the peanut kefir medium was between 3.44 - 4.12. Peanut kefir with tape yeast inoculum meets the standard requirements for fermented milk and can replace milk kefir.

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### Introduction

Kefir is a fermented milk product that has a specific taste as a result of the fermentation of lactic acid bacteria and yeast that live together and are mutually beneficial. These bacteria play a role in acid formation, while yeast produces alcohol and carbon dioxide (Hasruddin & Pratiwi, 2015). In addition to the content of good bacteria and yeast, kefir also contains vitamins, minerals, essential amino acids that help maintain and improve body functions (Julianto et al., 2016). The nutritional content of curd kefir has been researched, curd kefir contains 0.7% lactic acid, 1.4% protein, 2.30% fat, and 3.15% carbohydrates (Kurniati et al., 2020). Kefir contains essential minerals and amino acids

that can repair damaged cells and contains calcium and magnesium (Surono, 2004). Kefir has very high benefits for the body besides obtaining good nutritional value it also provides good digestion because it can inhibit the growth of pathogenic bacteria. Besides being beneficial for the body with the development of cosmetic technology, kefir can be used as a skin lightening agent, anti-acne agent, as an antioxidant and as a wound healing agent (Dewi et al., 2018).

In general, the raw materials used in kefir production are animal milk, namely cow's milk and goat's milk. However, the availability of animal milk is limited and it is relatively expensive. In addition, there are also some people who are allergic to animal milk, so this

requires an alternative to substitute for animal milk, namely vegetable milk. Milk from the legume plant can be used as a base for making kefir. Kefir derived from peanut milk has the advantage of relatively affordable raw material prices and is more abundant than fresh milk, and has a nutritional value that is almost equal to the nutritional value of fresh milk. Vegetable milk used in kefir fermentation can come from legumes in the form of green beans, red beans, soybeans, peanuts or *Mucuna pruriens* (Pratitaningsih & Suryani, 2019).

Peanuts are a type of nuts that can be used as an alternative to vegetable milk because peanuts are rich in fat, high protein, iron, vitamin E, vitamin B complex, phosphorus, vitamin A, vitamin K, lecithin, choline and calcium (Pusat Data dan Sistem Informasi Pertanian, 2013; Rahmiana & Ginting, 2012). Peanut seeds contain 40-48% oil, 25% protein and 18% carbohydrates (Kumar et al., 2014; Santosa, 2010).

The type of kefir starter inoculum is one of the factors that affect the quality of kefir. The quality of kefir includes total acid content, pH, protein and organoleptic (color, aroma, taste, texture, and acceptability). The starter inoculum plays an important role in the fermentation process. Kefir starter is a mixture of lactic acid bacteria and yeast inoculum. *Lactobacillus kefirianofaciens* presented in all kefir grains at important levels and *Enterobacter*, *Acinetobacter*, *Enterococcus* and *Pseudomonas* sp. were observed in traditional kefir grains. The fungal microflora of kefir grains was dominated by yeast species and Dipodascaceae family was dominant and *Saccharomyces cerevisiae* presented in all kefir grains. Other yeast species belonging to Kazachstania, *Candida*, *Issatchenkia* and *Rhodotorula* species were also detected in kefir grains (Dertli & Çon, 2017). In this kefir, there are lots of microorganisms such as *Lb. brevis*, *Lb. kefir*, *Lb. acidophilus*, *Lb. bulgaricus*, *Lb. casei*, *Lb. kefirianofaciens*, *Lb. helveticus*, *Lactococcus lactis subsp. lactis*, and *Lactococcus lactis subsp. cremoris*. Yeast was isolated from kefir were identified as *Kluyveromyces marxianus*, *Kluyveromyces wickerhamii*, *Saccharomyces cerevisiae*, *Pichia angusta*, *Pichia guilliermondii*, *Candida glabrata*, *Kluyveromyces marxianus*

was identified in the isolates from all the kefir samples, followed by *Kluyveromyces wickerhamii*, *Pichia angusta*, *Pichia guilliermondii*, *Candida glabrata* (Setyowati & Setyani, 2016).

Kefir grains are very easy to experience inactivity so that it requires maintenance of kefir grains that must be carried out continuously in order to ensure the quality of kefir grains. In addition, kefir grains are difficult to obtain in some areas in Indonesia. Therefore, a source of lactic acid bacteria and yeast is needed other than kefir grains. Tape yeast is an inoculum that is widely applied to traditional Indonesian foods, such as cassava tape, black sticky rice tape, white sticky rice tape, peuyeum, and breum so that tape yeast is easily available throughout Indonesia.

In tape yeast there are lactic acid bacteria, including *Pediococcus pentosaceus*, *Enterococcus faecium*, *Lactobacillus curvatus*, *Weissella confusa* and *Weissella paramesenteroides* which are capable of forming lactic acid. In addition, there is also yeast *Saccharomyces* (Sujaya et al., 2002). Cow's milk kefir using tape yeast inoculum as a starter for the formation of kefir obtained a total LAB of  $1.1 \times 10^9$  cells/mL with a fermentation time of 24 hours and a total yeast at 48 hours fermentation obtained  $1.3 \times 10^6$  cells/mL (Sinurat et al., 2018). Tape yeast can also be used as a starter for fermenting cow's milk into yogurt (Oktaviana et al., 2015).

Giving an increased starter concentration will have an effect on the increase of lactic acid bacteria but cause the pH to be low. The concentration of starter plays a role in the conversion of lactose into lactic acid, so that giving a high concentration of starter yeast will also produce high lactic acid (Agustina et al., 2013). The length of fermentation also affects the quality of the kefir. The longer the microbial fermentation time will multiply and the ability to break down the glucose substrate into alcohol is getting bigger (Kunaepah, 2008). The longer the fermentation time, the lower the taste preference for kefir (Purbasari et al., 2013). The quality of kefir in accordance with the SNI has a total acid of around 0.5% - 2%, a pH of 4.6 and a protein content of 3.2% (Zakaria, 2009).

Based on this, this study aims to determine whether peanut milk can be converted into kefir with the help of tape yeast. In addition, to determine the quality of peanut milk kefir, the difference in the percentage of starter yeast tape and the length of fermentation time was used.

### Materials and Methods

Materials used were petri dishes, erlenmeyer flasks, volumetric flasks, tubes, volumetric pipettes, autoclaves, ovens, laminar air flow, alcoholmeter, vortex, water bath, incubators, burettes, bunsen, hot plates, cotton swabs, alcohol tables, ragi tape, de Man Ragoza and Sharpe Agar (MRSA) media, Potato Dextrose Agar (PDA), alcohol, distilled water, peanuts, NaOH, phenolphthalein. The research method is arranged in an experiment with experimental methods. The stages in this research are:

#### Making Peanut Juice

The procedure for making peanut juice (*Arachis hypogaea* L.) according to the modified method Khairunnisa (2014). Peanuts were sorted by separating rotten nuts from the remaining soil, dust and other impurities. Peanut seeds were removed from the shell. Peanuts were weighed as much as 2 kg. Soaked peanuts using water for 18 hours with a ratio of 2 kg of beans: 6 liters of water. The peanuts were drained, peeled and washed with clean water. Ground peanuts using a blender with a ratio of water: beans (8 liters of water: 1 kg of peanuts). The resulting peanut slurry was filtered to extract the peanut filtrate. Peanut milk of 300 mL was added with 12 g of D-glucose (4% (w/v)) and pasteurized at 80 °C for 15 minutes.

#### Fermented Peanut Kefir

Procedure for making peanut kefir according to the modified Sinurat et al. (2018) method. A total of 300 ml of peanut milk and

tape yeast with varying concentrations of 2 gram (1% (w/v)), 6 gram (2% (w/v)) and 12 gram (4% (w/v)). Kefir fermentation was carried out for up to 72 hours with observations made at 24, 48, and 72 hours incubation. Shaking was carried out for 10 minutes on an orbital shaker at a speed of 100 rpm at 24, 48, and 72 hours incubation.

#### Determination of Total LAB

Determination of the total LAB was carried out using the total plate count method. The dilutions used are  $10^{-5}$  and  $10^{-6}$ . Each 1 ml sample was taken and then transferred to a petri dish. The sterile de Man Ragoza and Sharpe Agar (MRSA) medium was inserted into a petri dish. Then the media and the suspension were leveled by shaking the cup (pour plate method). Then incubate for 24 hours at 37 °C. Then the colony count was carried out (Sinurat et al., 2018).

#### Determination of Total Yeast

The media used was PDA (Potato Dextrose Agar). The dilutions used are  $10^{-5}$  and  $10^{-6}$ . Each 1 ml sample was taken and then transferred to a petri dish. A total of 15 ml of PDA media was added to the petri dish. Incubation was carried out at room temperature for 48 hours. Then the observations were made by counting the number of colonies (Sinurat et al., 2018).

#### Determination of Total Acid

As much as 10 ml of peanut juice was put into a volumetric flask of 100 mL and distilled water was added to the limit. 10 mL of the sample were taken and put into Erlenmeyer then added 2-3 drops of 1% phenolphthalein as an indicator. Then the titration was carried out with 0.1N NaOH while shaking it until a stable pink color was formed. After that the usage of the titer is recorded and the acidity of the kefir is calculated as percent acid (Sinurat et al., 2018).

$$\text{Total Acid (\%)} = \frac{(\text{Vol NaOH} \times \text{N NaOH} \times \text{FP} \times \text{MW acid})}{(\text{Sample Volume (mL)})} \times 100\%$$

Information: N NaOH (0.1 N); FP (10); MW acid (90); Sample volume (10 mL)

## pH and alcohol measurement

pH measurement using a pH meter and measurement of pH using an alcoholmeter (Sinurat et al., 2018).

## Results and Discussion

### Analysis of Total Lactic Acid Bacteria

Peanut juice inoculated with ragi tape at concentrations of 1%, 2% and 4% experienced changes in microbiology and biochemistry. Increased populations of lactic acid bacteria (LAB) and ragi tape were able to use peanut juice as a metabolic substrate. Based on the calculation of the total LAB for 72 hours, there was a change in the number of BAL. At an inoculum concentration of 1% and 24 hours of fermentation, the total LAB obtained was 8.08 log cells/mL ( $1.2 \times 10^8$  cells/mL) and increased to 8.28 log cells/mL ( $1.9 \times 10^8$  cells/mL) at the time of fermentation for 48 hours. However, it decreased at 72 hours of fermentation to 8.15 log cells/mL ( $1.4 \times 10^8$  cells/mL). Likewise, the 2% ragi tape inoculum increased up to 48 hours and decreased in the fermentation time of 72 hours. The 24 hours fermentation obtained a total LAB 8.11 log cells/mL ( $1.3 \times 10^8$  cells/mL), 48 hours fermentation time obtained 8.40 log cells/mL ( $2.5 \times 10^8$  cells/mL) and fermentation time 72 hours to 8.20 log cells/mL ( $1.6 \times 10^8$  cells/mL). Whereas in peanut kefir with 4% ragi tape inoculum, the total LAB decreased from 24 hours to 72 hours. The 24-hour fermentation time with 4% ragi tape inoculum obtained a total LAB of 8.64 log cells/mL ( $4.4 \times 10^8$  cells/mL), 48 hours of fermentation of 8.48 log cells/mL ( $3.0 \times 10^8$  cells/mL) and becomes 7.99 log cells/mL ( $9.8 \times 10^7$  cells/mL) at the 72-hour fermentation time (Figure 1).

From the results obtained, it could be seen that the addition of ragi tape inoculum affects the total LAB in the process of kefir. The decrease in the amount of LAB in the fermentation time from 48 hours to 72 hours with a ragi inoculum concentration of 1% and 2% and a ragi inoculum concentration of 4% has decreased from 24 hours due to limiting factors, namely nutrition so that there is competition between LAB and LAB or yeast.

The graph in Figure 1 shows the growth phases of LAB, namely the exponential phase, and the death phase. The growth phase of bacteria consists of a lag phase, an exponential

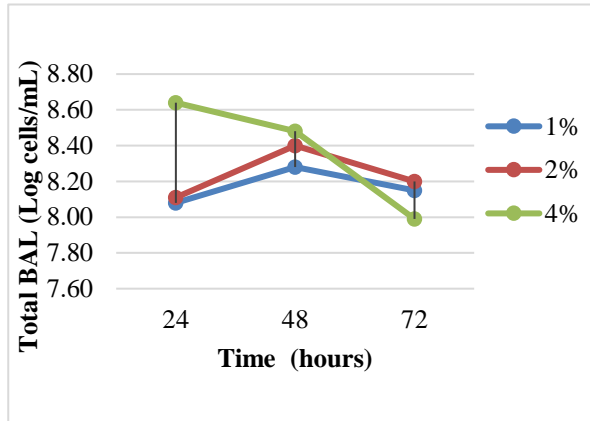
phase, a stationary phase and a death phase. In the lag phase, the increase in the number of bacteria takes place slowly, this is because the bacteria are in the process of acclimatization to environmental conditions (pH, temperature and nutrients). The lag phase in the research potential LAB is not observed because the observed time has a long time span. The next phase is the exponential phase which is a phase where bacterial growth takes place very quickly. In the growth of LAB isolates, the exponential phase occurred at 24 to 48 hours. The next phase was the stationary phase, in this phase there was no addition of bacteria because the number of growing cells was the same as the number of dead cells. The stationary growth phase and the death phase occurred from the 48 hours to the 72 hours. This decrease was due to nutrients in the media and energy reserves starting to run out (Mardalena, 2016).

The use of ragi tape inoculum in the becoming of peanut kefir uses a type of homofermentative lactic acid bacteria. Homofermentative bacteria do not produce alcohol and cannot withstand the accumulation of alcohol produced by yeast, causing a decrease in the total LAB. The accumulation of alcohol produced by yeast causes damage to the bacterial cell walls. Lactic acid bacteria are included in the gram-positive type which have a thick cell wall composed of a peptidoglycan layer consisting of protein, teichoic acid and polysaccharides and the outer layer is surrounded by a layer of sulfur protein (Delcour et al., 1999).

The teichoic acid in the negatively charged cell walls will react with the alcohol in the media, causing dehydration of the cell walls. Dehydration causes the pores to shrink and there is a decrease in cell wall permeability (Fardiaz, 1992). Gram-positive bacteria are dominated by thick peptidoglycan up to 90%. The peptidoglycan compound is polar so it is easily soluble in ethanol. Alcohol with levels of 60-70% has inhibitory activity from the test microbes which are gram-positive. This inhibition is effective in these bacteria due to the thick layer of peptidoglycan easily soluble in alcohol (Rini & Nugraheni, 2018).

The content of peanuts is a polysaccharide and oligosaccharide that will form a gel during

the fermentation process, as a result the texture of the milk becomes thicker (Pramono, 2009). This is thought to complicate the metabolic process of LAB and the growth of LAB in using lactose, so that the growth of LAB will decrease and the lactic acid formed will be reduced.



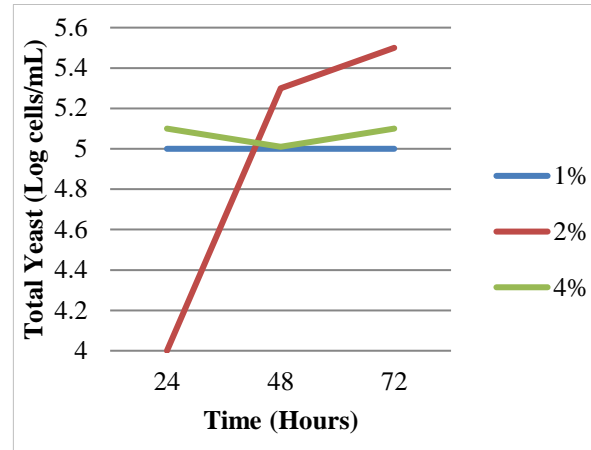
**Figure 1. Total Lactic Acid Bacteria in Peanut Kefir with Variation of Tape Yeast Inoculum**

**Total Yeast and Alcohol Content Analysis**

Yeast that lives on media containing glucose will use it as an energy source through the glycolysis process. If the growth is in aerobic conditions, after the glycolysis stage, the results of glycolysis, namely pyruvic acid will be converted into acetyl Co-A in the oxidative decarboxylation stage and proceed to the krebs cycle stage which produces a lot of energy used for cell work systems, cell organelle synthesis and to form generations (Kim & Gadd, 2008). However, if the growth conditions are anaerobic, pyruvic acid is converted into CO<sub>2</sub> and acetaldehyde which are then converted into alcohol and energy in smaller amounts (Drapcho et al., 2008).

Giving a different amount of inoculum has no effect on the total yeast. The total yeast was between 4 log cells/mL (1 x 10<sup>4</sup> cells/mL) - 5.5 log cells/mL (3 x 10<sup>5</sup> cells/mL) (Figure 2). The amount of inoculum also had no effect on alcohol content <1%. The addition of 1%, 2% and 4% inoculums produces almost even alcohol, another possibility because the fermentation conditions are the same, namely anaerobic, this is according to Buckle et al. (1985) that lactic acid bacteria generally produce large amounts of lactic acid from substrate fermentation carbohydrate energy. When grown in anaerobic environment, most

yeasts tend to ferment the carbohydrate substrate to produce ethanol along with a few other end products. Even so, the alcohol content in peanut kefir with tape yeast inoculum is in accordance with the opinion of (Surono, 2004) that the alcohol content of kefir is 0.5% - 1.0%.



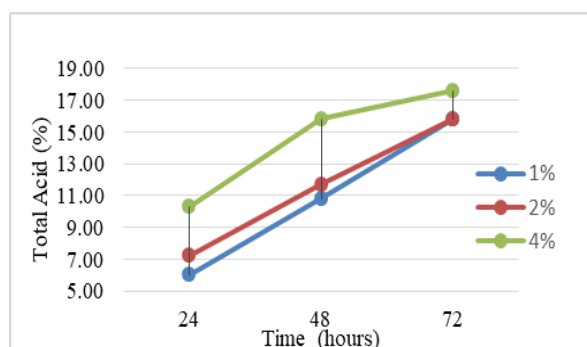
**Figure 2. Total Yeast in Peanut Kefir with Variation of Tape Yeast Inoculum**

**Total Acid Analysis**

The total acid in kefir drinks is the amount of lactic acid formed during the kefir fermentation process. Acid production is controlled by bacteria, while yeast produces alcohol (Jay et al., 2005). Total acid is the amount of acid formed during the fermentation process from the breakdown of lactose by LAB (Magalhães et al., 2011). During growth, LAB produces lactic acid which causes the pH to continue to decrease and this condition causes an environment that is not suitable for LAB while the acidic environment conditions support the growth of yeast. According to Winarno and Fernandez (2007), microbial growth in milk is related to the total amount of acid. LAB can grow optimally in total acid conditions < 2.5%.

The total acid in the fermentation of peanut kefir with ragi tape inoculum showed an increase. Total kefir acid of peanut inoculum ragi tape 1% with fermentation time of 24 hours, 48 hours and 72 hours, namely 6%, 10.8% and 15.8%. The total acid in the inoculum was 2% with a fermentation time of 24 hours, 48 hours and 72 hours, namely 7.2%, 11.7% and 15.8%. While the total acid in the inoculum was 4% with fermentation time of 24 hours, 48 hours and 72 hours, namely 10.3%, 15.8% and 17.6% (Figure 3). From these

results, it can be seen that the more inoculum was added and the longer the fermentation time of peanuts juice causes the total acid accumulation to be higher. The total yield of this acid is proportional to the decrease in the pH of the media.



**Figure 3. Total Acid in Peanut Kefir with Variation of Tape Yeast Inoculum**

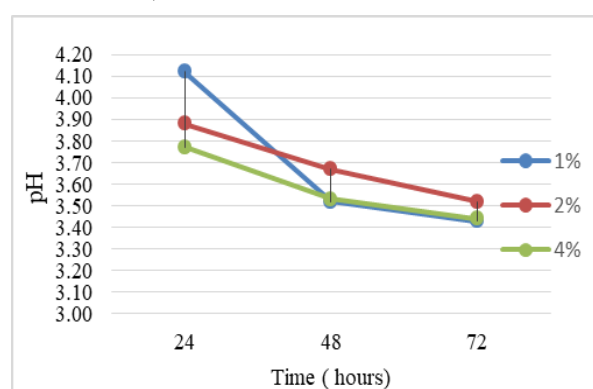
### pH Value Analysis

The degree of acidity (pH) is one of the important factors that need to be considered in fermented drinks. The pH of fermented beverage products is influenced by organic acids such as lactic acid, acetic acid, butyric acid and propionate acid produced during fermentation. pH is a measure of the hydrogen ion concentration of a solution. A pH measurement will reveal if a solution is acidic or basic. The pH tool measures the concentration of  $H^+$  ions released. The pH and acidity values are very important in kefir products because they will affect the growth of the microbial population, because the acid produced will inhibit microbial growth.

In the research of peanut kefir with tape yeast inoculum, the total acid yield continued to increase, which means that the  $H^+$  ion also increased, causing a decrease in pH (Figure 4). Peanut kefir with 1% inoculum decreased pH from 4.12 at 24 hours of fermentation to 3.43 at 72 hours of fermentation. The 2% inoculum decreased pH from 3.88 at 24 hours of fermentation to 3.52 at 72 hours of fermentation. Kefir with 4% tape yeast inoculum decreased pH from 3.77 during 24 hours of fermentation to 3.44 at 72 hours of fermentation. This happens because the longer the fermentation takes place, the LAB activity increases, causing the pH value to decrease. Lactic acid bacteria with the longer fermentation time have more time to break down carbohydrates and proteins which are

used as energy sources for LAB growth and produce organic acids as by products that can lower the pH of the media.

The results of measuring the pH of peanut kefir with tape yeast inoculum are not different from the pH of kefir with tape yeast inoculum and the basic ingredients of cow's milk were 4.37-6.30 (Sinurat et al., 2018), goat milk was 4.8 (Kurniati et al., 2020). Etawa goat's milk kefir with kefir seed inoculum has a pH of 4.57 (Pamericar et al., 2018), a combination of cow's milk and soy milk kefir has a pH of 3.96-4.76. Peanut kefir has a pH of 4.3 (Santos et al., 2014). Walnut kefir has a pH of 4.16 (Cui et al., 2013).



**Figure 4. The pH of Peanut Kefir Media with Variation of Tape Yeast Inoculum**

The standard composition of milk kefir was Lactic acid bacteria at least 7 log cells/ml ( $10^7$  cells/ml), yeast at least 4 log cells/ml ( $10^4$  cells/ml), alcohol not stated and a minimum total acid of 0.6% (Codex Alimentarius Commission, 2003). Adams and Moss (2008) stated that the alcohol content of kefir varies between 0.01% and 1%. Standard determination of pH on kefir  $\pm$  3.7 (Kurniati et al., 2020). In this study peanut kefir, the amount of LAB was 7.99 log cells/mL ( $9.8 \times 10^7$  cells/mL) - 8.64 log cells/mL ( $4.4 \times 10^8$  cells/mL), the amount of yeast was 4 log cells/mL ( $1 \times 10^4$  cells/mL) - 5.5 log cells/mL ( $3 \times 10^5$  cells/mL), alcohol content <1%, total acid obtained between 6% - 17.6% and the pH of peanut kefir between 3.44 - 4.12. Peanut kefir with ragi tape inoculum meets all composition standards.

### Conclusion

The following conclusions can be drawn: Peanuts can replace milk as a raw material for making kefir and tape yeast can be used to

ferment peanut milk. Out of several factors tested, fermentation time had a pronounced effect on the quality of the beverage. The suggested optimum fermentation conditions according to our experiments are the following: fermentation time of 12 hours, inoculum size of 2 g of tape yeast /100mL of peanuts milk. Peanut kefir with yeast tape starter inoculum can be developed as a source of active ingredients in cosmetics, which milk-based kefir has been widely developed in cosmetic products.

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