Running title: Stability of Grain Kefir Tablets During Storage

**Stability of Grain Kefir Tablets During Storage At Room Temperature for Three Weeks**

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**Novelty Statement**

Kefir grains have wet and sticky characteristics. Kefir grains can be modified into tablet to improve shelf life in room temperature and easily to distribute. These results show that grain kefir tablets have a good stability in room temperature during storage.

**Abstract**

Kefir grains are a probiotic product in the form of wet granules. Handling and storing kefir grains are less effective because they must be refreshed periodically and stored at low temperatures. Microencapsulation technology is used to produce more practical starter kefir tablets. This study aims to determine the stability of grain kefir tablets for three weeks of storage at room temperature. The produce of grain kefir tablets is carried out using the dry granulation method (slugging) to produce tablets with a diameter of 0.2 mm. Starter kefir tablets are packaged in zip lock plastic, put into a lock and lock container, and then put into 35 L containers for three weeks at room temperature. Starter kefir tablets were tested for water activity using an aw meter, water content using the thermogravimetric method, and viability of lactic acid bacteria using the total plate count method on days 0, 7, 14, and 21. The results showed that the water activity, water content, and viability of lactic acid bacteria of grain kefir tablets decreased with increasing storage time. Grain kefir tablets were still of good quality during three weeks of storage.

**Keywords:** Starter kefir tablets, lactic acid bacteria, water content, water activity

**Introduction**

Kefir is produced through a fermentation process by microorganisms found in kefir grains. Several types of microorganisms found in kefir grains include lactic acid bacteria (LAB), acetic acid, and bacteria and yeast (Erten *et al.,* 2014; Garofalo *et al.,* 2020; Chen *et al.,* 2021; Wang *et al.,* 2022). All microorganisms in kefir grains will utilize the substrate as lactose and glucose in skim milk to be converted into lactic acid.

 Microorganisms found in kefir grains have the potential as probiotics. The requirement for a product to be said to be a probiotic product is to have a minimum number of live microorganisms of 107 colonies/gram (Shori 2016; Ranadheera *et al.,* 2016; Pane *et al.,* 2018). An organism is said to be a probiotic if it can pass through gastric juice and symbiosis with the existing microflora in the intestine.

 Kefir grains are shaped like cauliflower, yellowish-white in color, and have a sticky texture. Kefir grains vary in size from 0.3 cm to 3.5 cm (Plessas *et al.,* 2016). Kefir grain-producing areas will affect the size of the kefir grains as well as the number and types of microorganisms in the kefir grains. Kefir grains preparation in the form of wet granules require an activation procedure that must be carried out periodically. The activation procedure aims to keep the microorganisms in the kefir grains alive (Hong *et al.,* 2019; Wang *et al.,* 2021). Kefir grains must also be stored in cold temperatures to keep the microorganisms alive. Microorganisms in kefir grains are in a state of dormancy during the storage period. Therefore, research is needed to create kefir grains in simpler preparations to facilitate use and storage. Tablet preparations are easier to store and handle because they have good stability.

 Tablet form can be used into dietary supplement so that, it can be consumed by humans every day. A good grain kefir tablet should still be able to maintain the viability of LAB at a certain standard. This research aims to evaluate the storage stability of grian kefir tablets during storage.

**Materials and Methods**

**Materials**

The materials used in this study consisted of materials for making starter kefir tablets and testing materials. The material for making starter kefir tablets is powdered kefir starter as the active ingredient; Sodium alginate (Buana Chem Bandung, Indonesia), Magnesium Stearate (Pharmapreneur Store Depok, Indonesia), Hydroxypropyl Methylcellulose (CV Aloin Labora Kediri, Indonesia), povidone (PVP) K-30 (CV Aloin Labora Kediri Indonesia), and talc (CV Aloin Labora Kediri Indonesia). The material needed for testing starter kefir tablets is de Man Rogosa Sharpe Agar (MERCK, Germany).

**Production of grain kefir tablets**

Tablets were made by the dry granulation method (Baseer et al., 2013). All ingredients were weighed according to a predetermined formulation in table 1. All ingredients, except sodium alginate and magnesium stearate, were mixed. The mixed material is then compressed with a tablet press to form slugging. The tablets that have been formed are then crushed. The granules are added with sodium alginate, and magnesium stearate, then mixed and sieved using a sieve (mesh size 40). The material is then compressed again to produce kefir starter tablets with a diameter of 0.2 mm.

**Storage of grain kefir tablets**

The resulting starter kefir tablets were then put into four different ziplock and labeled for days 0, 7, 14, and 21. And then, the entire ziplock is put into a 350 mL plastic lock and lock container. The lock and lock container is then put into a 20 L plastic container and placed at room temperature.

**Analysis of water activity (aw)**

Water activity testing was carried out using an aw meter (Lian *et al.,* 2015). The kefir grain tablet sample was put into the aw meter and waited until the water activity value was displayed on the screen.

**Analysis of water content**

The water content was tested gravimetrically using oven drying at 105°C (Souza *et al.,* 2017). The water content testing method begins with an empty porcelain cup in the range at 105°C for 1 h. The baked porcelain cup was then placed in a desiccator for 15 min and weighed as the weight of the empty cup. A sample of 2 g (W1) was put into an empty porcelain cup and then baked in the oven at 105°C for 4-6 h. Samples that have been baked are then placed in a desiccator for 15 min and weighed to record the weight. The sample was baked again until a constant weight (W2) was achieved, which is the difference in weight was less than 0.02 mg (Ramadhani *et al.,* 2017). The water content of the sample can be calculated using the following formula:

$$Water content\left(\%\right)=\frac{W\_{1}-W\_{2}}{W\_{2}}x100\%$$

**Analysis of the viable number of Lactic Acid Bacteria**

Analysis the viable numbers of Lactic Acid Bacteria by plate count method (Mandang *et al*., 2016). 1 g of sample was dissolved in 9 ml of 0.85% NaCl and calculated as a 10-1 dilution level. Dilutions were made up to a dilution level of 10-6. Samples from the last three levels of dilution were taken as much as 1 ml and then put into a sterile petri dish. Liquid MRSA media as much as 15-20 ml is poured into a petri dish. All petri dishes were placed in an incubator at 37°C for 48 hours. The number of viable colonies will be calculated using the following formula:

$Σ$ Colonies $=\frac{1}{dilution factor}$

**Statistical analysis**

Design of the experiments was completely randomized with five replication. Data were analyzed using the ANOVA method to determine the effect of treatment. 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. Data were statistically analyzed using SPSS 26.0 computer software.

**Result**

Analysis of variance (ANOVA) showed that storage time had a significant effect on water content and water activity (Table 1), and also significantly affected viability of Lactic Acid Bacteria (Table 2).

**Physical analysis**

**Table 1**: Analysis of Variance (ANOVA) of the effect storage time grain kefir tablets

|  |  |  |
| --- | --- | --- |
| Day | Water Content (%) | Water Activity |
| 0 | 13,46±0,60a | 0,498±0,005a |
| 7 | 12,77±0,18b | 0,492±0,005a |
| 14 | 11,95±0,23c | 0,450±0,004b |
| 21 | 11,29±0,34d | 0,479±0,005c |

Information:

\*Data shown as the mean value of 5 replicates ± standard deviation

\*Different lowercase superscripts show a significant effect (P<0,05)

Based on Table Table 1 shows that the length of storage time has a significant effect (P<0.05) on water content. Starter kefir tablets experience a decrease in water content with increasing storage time. A reduction in relative humidity around the storage room causes a decrease in water content. Low relative humidity causes the product to lose its ability to absorb water (Fayose and Huan, 2016). This is by the data in table 1, which shows that the longer storage time causes the grain kefir tablets to absorb less environmental moisture. It causes the water content of the grain kefir tablets to decrease with increasing storage time.

**Table 2**: Analysis of Variance (ANOVA) of the effect of storage time in viable number of LAB

|  |  |
| --- | --- |
| Day | Viability of Lactic Acid Bacteria (CFU/g) |
| 0 | 5,98x107a |
| 7 | 1,48x107b |
| 14 | 1,27x107b |
| 21 | 3,23x106c |

Information:

\*Data shown as the mean value of 5 replicates ± standard deviation

\*Different lowercase superscripts show a significant effect (P<0,05)

Table 2 shows that the length of storage time has a significant effect (P<0.05) on the viability of LAB, indicating a decrease in the number of LAB cells as the storage time of starter kefir tablets increases. The viability of LAB produced on days 0, 7, 14, and 21, respectively, were 5.98x107 CFU/g; 1.48x107 CFU/g; 1.27x107 CFU/g; and 3.23x106 CFU/g. The decrease in the viability of LAB in starter kefir tablets was influenced by the length of storage time and storage temperature. Room temperature causes many microorganisms to die due to damage to the protective cell walls (Andrade *et al.,* 2019).

**Discussion**

Changes in relative humidity during storage can be affected by packaging materials (Chen *et al.,* 2019). Kefir grain tablet samples in this study were stored in plastic *ziplock* and then put in l*ock and lock* containers made of plastic. Plastic packaging that is tightly closed will allow the conditions of the storage room to become stuffy (Befikadu, 2014) resulting in an accumulation of heat which causes an increase in temperature in the storage room. Increasing the temperature of the storage room will cause the sample to experience a decreased water content (Syamaladevi *et al.,* 2016). This is by the data in table 1, which shows that sample day 21 has the lowest water content. It could be since on the 21st day; the storage room conditions had the highest temperature causing the sample to have a low relative humidity so that the sample would release the water contained and cause a lower water content. All of the produced starter kefir tablets have a water content range of 11.29-13.46%. The recommended water content to support the stability of probiotic efficiency in tablets is less than 4% (Gurram *et al.,* 2021).

Table 2 shows that the storage time of starter kefir tablets has a significant effect on the decrease in aw (P<0.05). The reduction in aw was influenced by the length of time the grain kefir tablets were stored. This is the opinion of Liao *et al.* (2021), which states that the long shelf life of probiotic tablets can reduce the aw of the product. According to Chochkov *et al.* (2018) the low aw value is caused by the freeze dryer process which combines vacuum conditions with low temperatures so that the water content and aw value will decrease more.

The decrease in aw in products is also influenced by the use of packaging materials in the form of plastic, which does not have high water-tightness and airtightness (Sarastuti and Yuwono, 2015). The decrease in aw on the product is also affected by storage temperature. The product is stored at room temperature, and temperature control is not carried out during storage. This allows for an increase in temperature, which can reduce the aw of the product (Pulungan *et al.,* 2016).

The aw value of starter kefir tablets is still in the aw range of 0.4, which allows the number of microorganisms to grow quite a lot even though the number has decreased (Succi, 2017). A good kefir tablet grain should have an aw <0.2 to maintain product stability during shelf life, including product viability during the shelf life (Liao et al., 2021).

The bacteria in the starter kefir tablets are alive so they are still carrying out their metabolic activities. During the shelf life, bacteria will utilize the substrate in tablet materials to carry out metabolic activities and produce organic acids and bacteriocins which can reduce the effectiveness of the protective agent during the shelf life (Kumar, 2016; Andrade *et al.,* 2019).

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During the shelf life, it is possible to damage microbial cells due to lipid oxidation events in the material, so a good protective agent should have antioxidant activity to inhibit lipid oxidation in microbial cells (Kim *et al.,* 2021). The water greatly influences the viability of LAB in starter kefir tablets and aw content of the tablets during their shelf life (Jimenez *et al.,* 2015; Dias *et al.,* 2018).

The shelf life simulation carried out on grain kefir tablets shows that he product can still have good LAB quality and quantity after being stored for three weeks. If this product is later commercialized and intended as a supplement, then the product is still said to have a good shelf life. The assumption is that in one strip there are ten tablets and each tablets is consumed every day so it only takes 10 days for the shelf life.

**Conclusion**

Storage of starter kefir tablets for three weeks significantly decreased water activity, water content, and viability of lactic acid bacteria. This study showed that the storage of starter kefir tablets for three weeks was still of good quality based on the viability of lactic acid bacteria, which still met the standards of probiotic products.

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**Author contributions**

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

**References**

Andrade DP, CL Ramos, DA Botrel, SV Borges, RF Schwan, DD Ribeiro. 2019. Stability of microencapsulated lactic acid bacteria under acidic and bile juice conditions. International Journal of Food Science & Technology 54(7): 2355–2362.

Baseer A, F Hassan, SMF Hassan, S Jabeen, F Israr, G Murtaza, N Haque. 2013. Physico-chemical comparison of famotidine tablets prepared via dry granulation and direct compression techniques. Pakistan Journal of Pharmaceutical Sciences 26(3): 439–443.

Befikadu, D. 2014. Factors affecting quality of grain stored in Ethiopian traditional storage structures and opportunities for improvement. International Journal of Sciences: Basic and Applied Research 18(1): 235–257.

Chen H, J Wang, Y Cheng, C Wang, H Liu, H Bian, Y Pan, J Sun, W Han. 2019. Application of protein-based films and coatings for food packaging: A review. Polymers 11(12): 1–32.

Chen Z, T Liu, T Ye, X Yang, Y Xue, Y Shen, Q Zhang, X Zheng. 2021. Effect of lactic acid bacteria and yeasts on the structure and fermentation properties of Tibetan kefir grains. International Dairy Journal 114: 1–35.

Chochkov R, G Gercheva, N Dimitrov. 2018. Effect of storage time on hardness and water activity of pastry biscuits with thyme, oregano, and sage. International Research Journal of Advanced Engineering and Science 3(1): 177–179.

Dias CO, JDSO de Almeida, SS Pinto, FC de Oliveira Santana, S Verruck, CMO Müller, ES Prudêncio, RDDMC Amboni. 2018. Development and physico-chemical characterization of microencapsulated bifidobacteria in passion fruit juice: A functional non-dairy product for probiotic delivery. Food Bioscience 24: 26–36.

Erten, H. 2014. Importance of yeasts and lactic acid bacteria in food processing. In Malik, A., Erginkaya, Z., Ahmad, S. and Erten, H. (Eds). Food Processing: Strategies for Quality Assessment, p. 351–378. New York: Springer New York, NY.

Fayose F, Z Huan. 2016. Heat pump drying of fruits and vegetables: Principles and potentials for Sub-Saharan Africa. International Journal of Food Science 2016: 1–16.

Garofalo C, I Ferrocino, A Reale, R Sabbatini, V Milanović, M Alkić-Subašić,  F Boscaino, L Aquilanti, M Pasquini, MF Trombetta, S Tavoletti, R Coppola, L Cocolin, M Blesić, Z Saric, F Clementi, A Osimani. 2020. Study of kefir drinks produced by backslopping method using kefir grains from Bosnia and Herzegovina: Microbial dynamics and volatilome profile. Food Research International 137(2020): 1–15.

Gurram S, DK Jha, DS Shah, MM Kshirsagar, PD Amin. 2021. Insight on the critical parameters affecting the probiotic viability during stabilization process and formulation development. AAPS PharmSciTech 22(5): 1–22.

Hong JY, NK Lee, SH Yi, SP Hong, HD Paik. 2019. Physicochemical features and microbial community of milk kefir using a potential probiotic *Saccharomyces cerevisiae* KU200284. Journal of Dairy Science 102(12): 10845–10849.

Jiménez M, E Flores-Andrade, LA Pascual-Pineda, CI Beristain. 2015. Effect of water activity on the stability of *Lactobacillus paracasei* capsules. LWT-Food Science and Technology 60(1): 346–351.

Kim SI, JW Kim, KT Kim, CH Kang. 2021. Survivability of collagen-peptide microencapsulated lactic acid bacteria during storage and simulated gastrointestinal conditions. Fermentation 7(3): 1–10.

Kumar A, D Kumar. 2016. Development of antioxidant rich fruit supplemented probiotic yogurts using free and microencapsulated *Lactobacillus rhamnosus* culture. Journal of Food Science and Technology 53(1): 667–675.

Lian F, W Zhao, RJ Yang, Y Tang, W Katiyo. 2015. Survival of *Salmonella enteric* in skim milk powder with different water activity and water mobility. Food Control 47: 1–6.

Liao Y, Y Hu, N Fu, J Hu, H Xiong, XD Chen, Q Zhao. 2021. Maillard conjugates of whey protein isolate–xylooligosaccharides for the microencapsulation of *Lactobacillus rhamnosus*: Protective effects and stability during spray drying, storage and gastrointestinal digestion. Food & Function 12(9): 4034–4045.

Meidistria TR, L Sembiring, ES Rahayu, N Haedar, Z Dwyana. 2020. Survival of *Lactobacillus plantarum* dad 13 in probiotic cheese making. IOP Conference Series: Earth and Environmental Science, p. 1–7. Boston: IOP Publishing.

Pane M, S Allesina, A Amoruso, S Nicola, F Deidda, L Mogna. 2018. Flow cytometry: evolution of microbiological methods for probiotics enumeration. Journal of Clinical Gastroenterology 52: S41–S45.

Plessas S, C Nouska, I Mantzourani, Y Kourkoutas, A Alexopoulos, E Bezirtzoglou. 2016. Microbiological exploration of different types of kefir grains. Fermentation 3(1): 1–10.

Pulungan MH, S Sucipto, S Sarsiyani. 2016. Penentuan umur simpan pia apel dengan metode ASLT (studi kasus di UMKM Permata Agro Mandiri Kota Batu). Industria: Jurnal Teknologi dan Manajemen Agroindustri 5(2): 61–66.

Ramadhani PD, BE Setiani, H Rizqiati. 2017. Kualitas selai alpukat (*Persea americana* Mill) dengan perisa berbagai pemanis alami. Jurnal Teknologi Pangan 1(1): 8–15.

Ranadheera CS, CA Evans, M Adams, SK Baines. 2016. Co-culturing of probiotics influences the microbial and physico-chemical properties but not sensory quality of fermented dairy drink made from goats’ milk. Small Ruminant Research 136: 104–108.

Sarastuti M, SS Yuwono. 2015. Pengaruh pengovenan dan pemanasan terhadap sifat-sifat bumbu rujak cingur instan selama penyimpanan [in press April 2014]. Jurnal Pangan dan Agroindustri 3(2): 464–475.

Shori AB. 2016. Influence of food matrix on the viability of probiotic bacteria: A review based on dairy and non-dairy beverages. Food Bioscience 13: 1–8.

Souza ACP, PD Gurak, LDF Marczak. 2017. Maltodextrin, pectin and soy protein isolate as carrier agents in the encapsulation of anthocyanins-rich extract from jaboticaba pomace. Food and Bioproducts Processing 102: 186–194.

Succi M, P Tremonte, G Pannella, L Tipaldi, A Cozzolino, R Coppola, E Sorrentino. 2017. Survival of commercial probiotic strains in dark chocolate with high cocoa and phenols content during the storage and in a static in vitro digestion model. Journal of Functional Foods 35: 60–67.

Syamaladevi RM, RK Tadapaneni, J Xu, R Villa-Rojas, J Tang, B Carter, S Sablani. B Marks. 2016. Water activity change at elevated temperatures and thermal resistance of *Salmonella* in all purpose wheat flour and peanut butter. Food Research International 81: 163–170.

Wang L, K Deng, Y Zhang. 2022. Isolation and screening of high‐quality lactic acid bacteria and yeast strains in kefir grains and preparation of kefir compound fermentation starter. Journal of Food Processing and Preservation46(11): 17073.

Wang X, W Li, M Xu, J Tian, W Li. 2021. The microbial diversity and biofilm-forming characteristic of two traditional Tibetan kefir grains. Foods 11(1): 1–19.