Yeast and lactic acid bacteria on kefir instant filled with flour of banana (Musa paradisiaca cultivar Mas)

R Azara*, I A Saidi, Giyanto

Department of Food Technology, UniversitasMuhammadiah Sidoarjo, Jl. Majapahit no 666B, Sidoarjo 61215, Jawa Timur, Indonesia

E-mail: rima.azara@gmail.com

Abstract. Kefir is a fermented milk drink produced by the actions of bacteria and yeast contained in kefir grains. It’s have a unique taste and unique properties. The industrial manufacture of kefir using grains as the starter culture is very difficult due to complexity of their microbiological composition, which varies widely depending on the origin of the grains and conditions of storage and handling. Instant kefir (powders) which produce in the research may provide the solution to extend the industrial manufacture of kefir. The purpose of this study was observed the effect of banana flour on yeast and lactic acid bacteria production. This study used descriptive method. Fermentation at room temperature for 48 hour to produced kefir. Instant kefir samples were prepared used of 10, 15, 20, 25, 30, 35, and 40% (w/w) banana flour, dried and than milled and passed through at 80-mesh. This research shows that instant kefir with 10% banana flours produce the highest yeast and lactic acid bacteria. Amount of microbial in the kefir are $8.8 \times 10^4$ cfu/ml yeast and $9.8 \times 10^5$ cfu/ml lactic acid bacteria.

1. Introduction
Fermented dairy products from milk are perhaps the most common fermented foods worldwide. Kefir is a fermented milk drink produced by the actions of bacteria and yeast contained in kefir grains, is reported to have a unique taste and unique properties. The grains have a specified structure and behave as biologically vital organisms. The microbial population of kefir grains consists of numerous species of lactic acid bacteria, yeast, and filamentous moulds which develop a complex symbiotic relationship within a microbial community. Kefir grains contains lactic acid bacteria (Lactobacillus, Lactococcus, Leuconostoc, and Streptococcus spp.) and yeast (Kluyveromyces, Saccharomyces, and Torula) included in a polysaccharide-protein matrix. The microflora of kefir grains is remarkably stable, retaining its activity for years if preserved and incubated under appropriate cultural and physiological condition [1, 2, 3, 4].

The industrial manufacture of kefir using grains as the starter culture is very difficult due to complexity of their microbiological composition, which varies widely depending on the origin of the grains and conditions of storage and handling. Instant kefir (powders) which produce in the research may provide the solution to extend the industrial manufacture of kefir [5, 6]. Thus, the objectives of this study were to develop an instant kefir powder product and evaluate the effect of banana flour of yeast and lactic acid bacteria production.

2. Experimental Method
Cow milk was obtain from Dairy Farm, Sidoarjo and pasteurized at 80 °C for 8 minutes, and then was cooled until 25 °C. The kefir grains as the starter, collected from homemade kefir in...
this study. The pasteurization-treated milk was inoculated with 5% (w/v) kefir grains and incubated at room temperature for 48 h. The kefir samples were analysed for physicochemical and microbial properties.

The preparation of banana flours (Musa paradisiaca cultivar Mas) from unripe banana. Banana obtained from Larangan tradisional market, Sidoarjo. The bananas were peeled and cut into slices of about 1 mm thickness. Slices were then dipped in 0.5% (w/v) citrate acid solution for 30 minutes, drained and dried in oven at 60-80 °C for 10 h. The dried samples were milled and passed through at 100-mesh.

Instan kefir was produced with combined banana flour and kefir. Instant kefir samples were prepared used of 10, 15, 20, 25, 30, 35, and 40% (w/w) banana flour. Then the samples dried in oven at 50 °C for 20 h. The dried samples were milled and passed through at 80-mesh. Instant kefir in this study was microbiological analysis including total analysis of lactic acid bacteria and yeast using TPC (Total Plate Count) method.

3. Results and Discussion

The Kefir samples were analyses for physicochemical and microbial properties. Table 1 shows the lactic acid bacteria, yeast, totals acid, pH, and viscosity properties. Kefir contains 5.5 x 10⁷ cfu/ml lactic acid bacteria and 9.3 x 10⁴ cfu/ml yeast. According to kefir standards, kefir which is prepared should higher than 10⁷ cfu/g total sum of specific microorganism which are lactic acid and acetic acid bacteria, higher than 10⁴ cfu/g yeast. Kefir in this research contains 1.88% total acid. According to kefir standards, kefir which is prepared higher than 0.6 titratable acidity which is expressed as % of lactic acid. [7] The pH value of kefir at 48-h of incubation reached of 3.84, that was in agreement with the pH values obtained by Pop et al.[8] Viscosity of kefir recorded in Table 1. is 169 cp.

Table 1. The physicochemical and microbial characteristics of kefir samples

| Parameters       | Kefir samples | Kefir (FAO/WHO 2001) |
|------------------|---------------|----------------------|
| **Microbiology** |               |                      |
| lactic acid bacteria | 5.5 x 10⁷ cfu/ml | > 10⁷                |
| yeast            | 9.3 x 10⁴ cfu/ml | > 10⁴                |
| **Chemical**     |               |                      |
| kefir susu sapi  |               |                      |
| total acid       | 1.88%         | > 0.6%               |
| pH               | 3.84          |                      |
| **Physic**       |               |                      |
| Viscosity (cp)   | 169 cp        |                      |

Kefir is a product elaborated from the symbiotic fermentation of different microorganisms. Kefir grains consist of lactic acid bacteria, acetic acid bacteria, yeasts, and other microorganisms. Best taste of kefir is ensured with a mixture of starter cultures producing all desired aroma active compounds. Different microorganisms growing together can lead to interaction among themselves. Table 2 shows the amount of lactic acid bacteria in instans kefir. Lactic acid bacteria are Gram-positive bacteria, which use carbohydrates as energy source by producing lactic acid [9, 10, 11].

Kefir is a natural probiotic. The probiotics are food products and nutritional supplements containing live microorganisms and other components of microbial cells that have an extremely beneficial impact on the host. Probiotics are foods that contain live bacteria, which beneficial to health such as improvement on digestive system, antimicrobial property,
antioxidant effect, betagalactosidase activity, lowering effects on cholesterols, anti-allergenic properties, anti-inflammatory activity, improvement in immune system, beneficial for lactose intolerance, and having antimutagenic effects.\[12, 13, 14\] Table 2 shows that instant kefir with 10% banana flour have $9.8 \times 10^5$ cfu/ml lactic acid bacteria. The kefir with 10% banana flour has the highest lactic acid bacteria level, and the lowest is 25% banana flour. Kefir consist of a complex set of lactic acid bacteria such as *Lactobacillus casei*, *Lactobacillus hilgardii*, *Lactobacillus delbrueckii spp bulbaricus*, *Lactobacillus plantarum*, *Lactobacillus kefir*, *Leuconostoc mesenteroides sp dextranicum* and *Sterptococcus lactis\[15\].

Kefir has a distinct flavour due to the presence of various compounds produced during the fermentation process. Lactic acid is the major metabolite produced by lactic acid bacteria. Other important metabolites produced are carbon dioxide ($CO_2$) and ethanol at low concentrations and flavour components such as acetaldehyde and acetone. The presence of ethanol is important for a kefir product because it confers the typical light alcoholic flavour and, together with the $CO_2$ mainly deriving from yeast fermentation, provides the final product with the desirable exotic notes and yeasty aroma \[14, 16\]. Yeasts are primarily responsible for the alcohol production in kefir \[17\].

**Table 2.** Mean counts (cfu/ml) of lactic acid bacteria in instant kefir

| banana flour : kefir (%) | LAB (cfu/ml) |
|--------------------------|--------------|
| 10 : 80                  | $9.8 \times 10^5$ |
| 15 : 75                  | $4.7 \times 10^4$ |
| 20 : 70                  | $9.1 \times 10^4$ |
| 25 : 65                  | $3.1 \times 10^4$ |
| 30 : 60                  | $6.4 \times 10^4$ |
| 35 : 55                  | $4.4 \times 10^4$ |
| 40 : 50                  | $3.9 \times 10^4$ |

Kefir also contains a variety of yeasts such as *Kluyveromyces marxianus*, *Kluyveromyces lactis*, *Naumovozyma sp.*, *Kazachastania kefir*, *Zygosaccharomyces spp.*, *Candida spp.*, *Candida lambica*, *Candida krusei*, *Candida lambica*, *Saccharomyces spp.*, *Saccharomyces bouardii*, *Saccharomyces humaticus*, *Pichia caribbica*, *Pichia fermentus*, *Torula* and *Cryptococcus spp.* Yeast strains such as *Saccharomyces bouardii* can also have probiotic activity; thus, they are considered probiotic-like microorganism. They are different from probiotics in that they are not found in the gastrointestinal tract of the host. The presence of *Saccharomyces cerevisiae* contributes to the enhancement of the sensory quality of the kefir beverage, promoting a strong and typically yeasty aroma, as well as its refreshing, pungent taste \[1, 14, 18, 19, 20, 21\]. Table 3 shows that the kefir with 10% banana flour has the highest yeast level, and the lowest is 25% banana flour.

**Table 3.** Mean counts (cfu/ml) of yeast in instant kefir

| banana flour : kefir (%) | yeast (cfu/ml) |
|--------------------------|----------------|
| 10 : 80                  | $8.8 \times 10^4$ |
| 15 : 75                  | $4.7 \times 10^4$ |
| 20 : 70                  | $5.2 \times 10^4$ |
| 25 : 65                  | $2.3 \times 10^4$ |
| 30 : 60                  | $6.7 \times 10^4$ |
4. Conclusion
This research shows that instant kefir with 10% Mas banana flour produced the highest yeast and lactic acid bacteria. Amount of microbial in the kefir are $8.8 \times 10^4$ cfu/ml yeast and $9.8 \times 10^5$ cfu/ml lactic acid bacteria.

5. Acknowledgements
We acknowledged UMSIDA (Grant: Program Hibah Penelitian dan Pengabdian Internal)

6. References
[1] Lengkey, H. A. W. & Balia, R. L. 2014 The effect of starter dosage and fermentation time on pH and lactic acid production Biotechnology in Animal Husbandry 30 2 339-347
[2] Edward, R. F. 2005 Kefir – a complex probiotic Food Science and Technology 2 1 1-17
[3] Simova, E., Beshkova, D., Angelov, A., Hristozova, Ts., Frengova, G., & Spasov, Z. 2002 Lactic acid bacteria and yeast in kefir grains and kefir made from them Journal of Industrial Microbiology & Biotechnology 28 1-6
[4] Pogacic, T., Sanja, S., Simun, Z., & Dubrauka, S. 2013 Microbiota of kefir grains Mljekarstvo 63 1 3-14
[5] Dadkhah, S., Rezvan, P., Mahnaz, M. A., & Ali, M. 2011 Kefir production from soymilk Annals of Biological Research 2 6 293-299
[6] Chia, H. C., Chin, W. L., & Ming, J. C. 2006 The Effect of freeze drying and rehydration on survival of microorganisms in Kefir Asian-Aust. J. Anim. Sci. 19 1 126-130
[7] Kahraman, C. 2011 Production of kefir from bovine and oat milk mixture Thesis Engineering and Sciences of Izmir Institute of Technology
[8] Pop, C., Sorin, A., Liana, S., Ancuta, M. RR., Marianne, S., Nicolas, M., & Carmen, S. 2014 Influence of different growth conditions on the kefir grains production, used in the kefir grains synthesis Bulletin UASVM Food Science and Technology 71 2 147-153
[9] Daniela, C., Neila, R., Patricia, V., Marcio, M., & Mauricio, R.C. 2017 Identification by PCR and evaluation of probiotic potential in yeast strains found in kefir samples in the city of Santa Maria, RS, Brazil Food Science and Technology
[10] Jasmin, S. 2013 Metabolic activity and symbiotic interaction of bacteria and yeasts in water kefir Dissertation
[11] Merih, K. 2015 Kefir as a probiotic dairy beverage: determination lactic acid bacteria and yeast International Journal of Food Engineering 1 155-60
[12] Otbes, S. & Ozlem, C. 2003 Kefir: A probiotic dairy-composition, nutritional and therapeutic aspects Pakistan Journal of Nutrition 2 2 54-59
[13] Gorsek, A. & Marko, T. 2011 Production of unique naturally immobilized starter: a fractional factorial design approach towards the bioprocess parameters evaluation Biomass – Detection, Production and Usage 185-200
[14] Mayumi, D. U. P. R., Joice, D. F. L. M., Thanise, S. S. S., & Ana V. B. M. 2014 Labneh with probiotic properties produced from kefir: development and sensory evaluation Food Science and Technology 34 4 694-700
[15] Habibi, N., Sabihe, S. Z., & Mahmoud, S. Z. 2011 Optimization of kefir grains
production by using taguchi technique and mini-fermentation World Applied Sciences Journal 12 5 613-618

[16] Corona, O., Walter, R., Alessandro, M., Rosa, G., Nicola, F., Huseyin, E., Giancarlo, M., & Luca, S. 2015 Characterization of kefir-like beverages produced from vegetable juice LWT-Food Science and technology 66 2016 572-581

[17] Ming-Ju, C., Je-Ruei, L., Chin-Wen, L., & Yu-Tzu, Y. 2004 Study of the microbial and chemical properties of goat milk kefir produced by inoculation ith Taiwanese Kefir Grains Institute of BioAgricultural Sciences, Academia Sinica, Taiwan, ROC 711-715

[18] Plessas, S., Chrysanthi, N., Ioanna, M., Yiannis, K., Athanasios, A., & Eugenia, B. 2016 Microbiological exploration of different types of kefir grains Fermentation 3 1 1-10

[19] Mei, J., Xin, G., & Yunfei, L. 2016 Kefir Grains and their fermented dairy products JSM Biotechnology & Biomedical Engineering 3 1 1-7

[20] Teixeira, K. M., Gilberto, V. M. P., Cassia, R. C., Giuliano, D., & Rosane, F. S. 2011 Brazilian kefir: structure, microbial communities and chemical composition Brazilian Journal of Microbiology 42 693-702

[21] Cecilia, B. S. A., Misael, C. R., & Olga, M. C. 2016 Identification of some kefir microorganism and optimization of their production in sugarcane juice Rev. Fac. Nac. Agron. 69 1 7935-7943