

SYNBIOTIC FUNCTIONAL DRINK FROM COW MILK FERMENTED WITH KEFIR AND SUPPLEMENTED WITH INULIN

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ABSTRACT

Increasing awareness on health has increased demand for healthy food confirmed by raising market sales of functional food. This study aims to develop a new synergetic drink from cow milk fermented by kefir grains and supplemented by inulin. Firstly, the inulin was isolated from dahlia flower tuber while fresh cow milk was incubated with kefir grains to produce kefir probiotic drink. Next, inulin with different concentrations was added to the probiotic drink and was fermented for 48 hours at room temperature generating a symbiotic functional drink. Analysis of antimicrobial properties revealed that synbiotic drinks have antimicrobial activity and the colony unit bacteria was met the standard to be claimed as a probiotic supplement. The microbiology and physicochemical properties of kefir synbiotic drink were analyzed and the result showed better properties than the control. There was no significant difference in taste between control and kefir enriched inulin drink assessed by the panelist. Only kefir synbiotic drink supplemented by inulin of 3 and 5% determined has a significant difference in aroma. Based on that kefir enriched inulin drink has the potency to be used as a functional drink that has a synergistic combination of prebiotics and probiotics.

Keywords: Kefir, Inulin, Synbiotic, Functional Food, Prebiotic, Probiotic.

RASĀYAN J. Chem., Vol. 15, No.1, 2022

INTRODUCTION

Demand for functional food has grown recently due to increasing interest in health.¹ Consumption of healthy food is reported could possibly reduce 20% of health expenditure per year per person.² In fact, more than 93% of people in North America eat healthy food as one strategy to prevent diseases.³ The consumption of healthy food is predicted to climb up to nearly 250 billion US\$ in 2024.⁴ The term functional food refers to a food or drink which has specific nutrients added to improve health or prevent diseases.⁵⁻⁷ The functional drink is the most popular functional food which is forecasted to have 7.8% market growth rate increment during 2017-2022.² Among functional beverages, the healthy drink containing probiotic substances has grown incredibly and accounted for 60-70% of total wellbeing life market products.⁸

Recently attempt has been made to combine probiotics and prebiotics with the addition of various bioactive compounds to produce a synergistic effect that can improve health.¹ Prebiotics are a short chain and low molecular polysaccharides which selective fermented in the colon. Prebiotics provide a suitable substrate for probiotics to grow in the large intestine.^{9,10} Therefore the addition of prebiotics in probiotics drink could create better colonic health which in addition could prevent some digestive diseases such as colon cancer, diarrheal, hypercholesterolemia, etc.^{1,11} Common prebiotics used in synbiotic drink including inulin, fructo-oligosaccharides, galacto-oligosaccharides, manno-oligosaccharides, xylo-oligosaccharides, trans-galacto-oligosaccharides, arabinosyl-oligosaccharides.¹ Inulin is a derivate of fructan consisting of fructose and glucose moieties which have been used in the food industry as gel-forming for improving viscosity and rectifying organoleptic properties and as the low-calorie sweetener.¹² Jerusalem artichoke, dahlia and chicory root are the main inulin source for industrial production.¹³ Inulin can also be found in some fruits and vegetables such as banana, onion, lettuce, garlic, leek, sunflower, and more.¹⁴

This work aims to develop a new synergetic drink enriched by different concentrations of inulin as prebiotics and lactic acid bacteria from the fermentation of cow milk with kefir grains. Inulin was isolated

from dahlia tubers which are abundant in Indonesia. The quality of the synbiotic drink was determined by testing the physicochemical and biological characteristics. In addition, the intent to buy was evaluated by asking about the aroma and taste of the synbiotic drink.

EXPERIMENTAL

Materials

Cow milk, dahlia tuber, and kefir grains were purchased from the local market in Medan – Indonesia. The chemicals used in this study were obtained from a local chemical supplier and were used without any purification.

Extraction Inulin From Dahlia Tuber

Isolation inulin from dahlia flower tuber was conducted following the previous procedure with slight modification.¹⁵ Firstly, the dahlia bulbs were peeled, cut and washed with water. Using a blender, 100 g of dahlia tuber was crushed with water in the ratio of 1:2 (w/v). Next, the dahlia pulp was heated for 30 min at a temperature of 80 - 90°C and allowed to cold. The filtrate was collected through separation and added ethanol 30% and stored at 0°C for 18 hours followed at room temperature for 2 hours until sediment appeared. The sediment was collected and dissolved with water. The procedure to obtain the sediment was repeated until pure inulin was collected. The wet pure inulin was dried in the oven at a temperature of 50 - 60°C for 7 hours and stored in a desiccator before use. The inulin was characterized using Fourier transform infrared (FT-IR).

Kefir Synbiotic Drink Preparation

The synbiotic drink was prepared by making probiotic kefir firstly. Fresh cow milk which has pasteurized was added by 5% (v/w) of kefir grains, stirred and incubated for 24 hours at room temperature. Afterward, the kefir grains were separated and 75 ml of kefir probiotic drink was used for making the synbiotic drink. For synbiotic drink, inulin with different concentrations of 3, 5, and 7% (v/w) was added to kefir and the solution was incubated for 48 hours at room temperature. A control group was also prepared following that procedure. Subsequently, the fermented synbiotic drink was analyzed.

Microbiology Analysis

The microflora of kefir was enumerated using agar plate count on MRS (de Man, Rogosa and Sharpe) medium for Lactobacilli and M17 medium for lactic streptococci while Yeast was calculated on PDA. The antimicrobial activity of the synbiotic kefir was evaluated using the previous method developed by Çadirci and Çitak.¹⁶

Physicochemical Analysis

The pH value was determined after fermentation using a pH meter while water content was measured using an oven drying method. The ASTM D5630 procedure was used to quantify ash content. The known amount sample was poured into pre-weighed crucible porcelain and burned in the furnace at a temperature of 500°C and allow to cool to room temperature. After that, the porcelain was weighed and the ash content result is expressed in percentage. The fat content was measured using the AOAC method and Kjeldahl method was used to estimate protein content.

Sensory Evaluation

To determine the intent of people to buy the kefir synbiotic drink, 25 untrained panelists (undergraduate students in the Department of Chemistry, University of Sumatera Utara) were asked to determine the aroma and taste. Panelists were recorded their perceptions using a five-point scale such as really like, like it, rather like it, don't like, and very dislike. Those Likert scales further were converted into a number and were reported using a radar plot graph.

Statistical Analysis

All data reported were analyzed using Statistical 13 software. The Tukey test was used to determine the difference of mean in this study. The effect was considered significant if have $p < 0.05$.

RESULTS AND DISCUSSION

Inulin Extraction and FT-IR Characterization

The extraction process of dahlia tuber using water and ethanol as solvent produced a polysaccharide named inulin with a yield of 1.86% based on its fresh weight. However, this yield is lower than the previous researcher obtained. Previous researchers extracted an inulin yield of 6.0 – 9.5% (w/w) from fresh dahlia tuber which has been planted for one year.¹⁵ Lower yield occurred in this study presumably due to immature dahlia tuber used in this study which was planted less than one year.

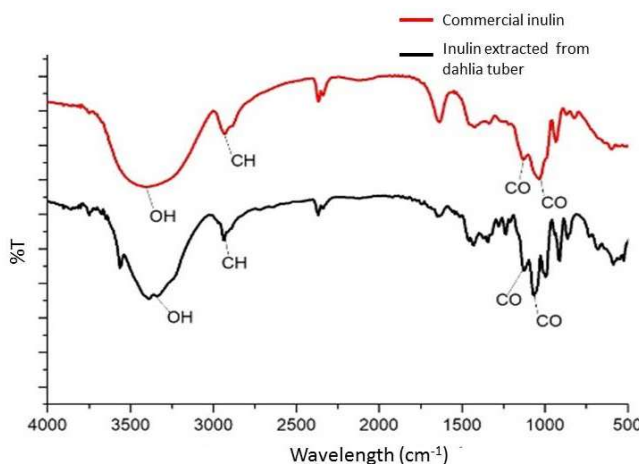


Fig.-1: FT-IR Spectra of Commercial Inulin and From This Study.

The extracted inulin was confirmed using FT-IR and the spectra were compared with commercial inulin which is available in the market. As can be seen in Fig.-1, both FT-IR spectra of commercial inulin and inulin extracted from dahlia tuber showed the same spectrum. Either commercial or extracted inulin displayed the absorption at a wavelength of $3340 - 3402 \text{ cm}^{-1}$ representing the stretching vibration of $-\text{OH}$ group. A weak and wide peak at a region of $2800 - 3000 \text{ cm}^{-1}$ was characterized by $-\text{CH}_2-$ group. Furthermore, all spectrums depict a vibration at a wavelength on a region of $1000 - 1200 \text{ cm}^{-1}$ which represents the appearance of the C-O group.

Synbiotic Preparation

The first step in making kefir drink was to pasteurize the cow milk at 65°C for 30 min. At this stage, the non-pathogenic bacteria particularly decomposing bacteria remain life. The addition of kefir grain followed by incubation overnight at room temperature was increased the probiotic bacteria. Inulin in different concentrations was added to the formed synbiotic drink after incubation for 48 hours at room temperature. The reducing sugar inversion test was conducted for glucose and fructose qualitative analysis. Figure-2A showed the result of the qualitative analysis.

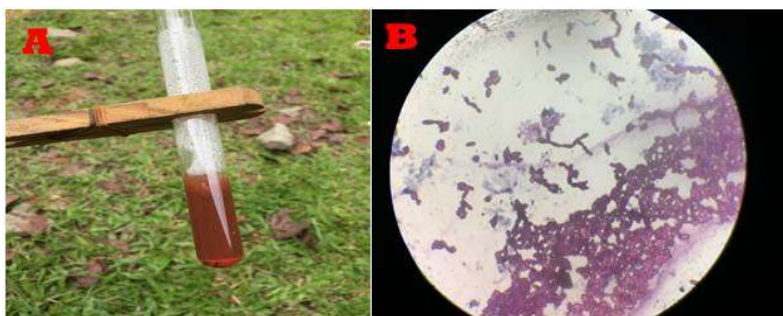


Fig.-2: The Qualitative (A) and Coloring Gram (B) Results of Kefir Synbiotic Drink.

Furthermore, the bacterial staining test result using a crystal violet-iodine complex as shown in Fig.-2B depicted purple color represents the appearance of gram-positive bacteria in forms of streptococcus. The gram-positive bacteria cell walls restrain the complex while the gram-negative organism decolorizes the complex rendering pink color.¹⁷

Microbiology Analysis

The agar diffusion test was used to determine the antimicrobial activity of kefir synbiotic drinks. The result obtained from clear zone examination (Fig.-3A) demonstrated that kefir added with inulin 3% have clear zone diameter of 8.3 mm while the addition of 5% and 7% showed a zone of 8.7 and 10.4 mm, respectively. Furthermore, as can be seen from Fig.-3B, synbiotic kefir drink with the addition of inulin 7% has the highest antimicrobial index compared to others. However, addition inulin has a significant effect either on clear zone area or antimicrobial index which has $p < 0.05$ calculated by the Tukey test. Therefore it can be concluded that increasing inulin in kefir drink could increase the antimicrobial activity of the synbiotic drink. This result revealed the opportunity of the kefir synbiotic drink to be used as an antimicrobial drink.

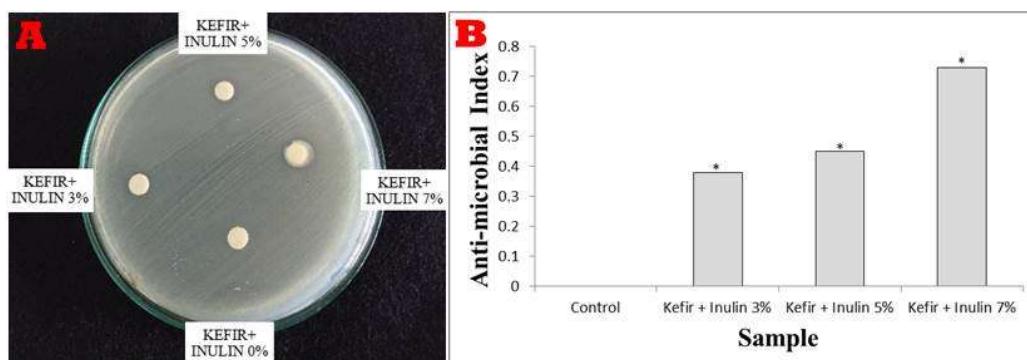


Fig.-3: The Clear Zone Area (A) and Antimicrobial Index (B) of Kefir Synbiotic Drink. A * symbol indicates a significant difference of mean determined by the Tukey test ($p < 0.05$).

As predicted the colony unit bacteria of kefir synbiotic drink with the addition of inulin presented a significant increase of CFUs / mL compared to the control as shown in Fig.-4. Kefir synbiotic drink with inulin treatment of 7% showed the highest colony unit bacteria of 67×10^9 CFUs/mL. These findings are in association with the previous study that concluded inulin as prebiotics provide good media for probiotic bacteria to grow.⁸

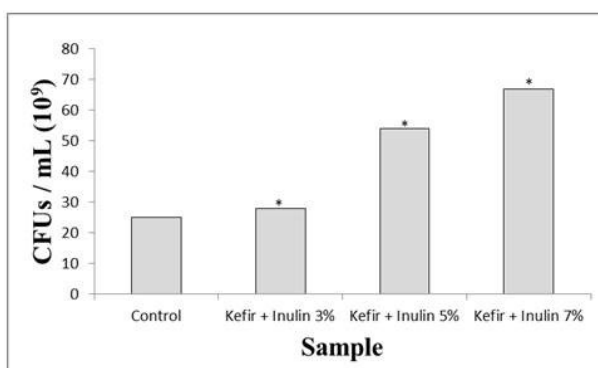


Fig.-4: The Colony Unit Bacteria of Kefir Synbiotic Drink. A * symbol indicates a significant difference of mean determined by the Tukey test ($p < 0.05$).

Physicochemical Analysis

The pH value of the kefir supplemented inulin drink was determined after incubation for 48 hours at room temperature. The pH values of the synbiotic drink are shown in Fig.-5A. As expected the pH values were increased in increasing inulin in the synbiotic drink. The kefir with an addition 7% inulin has the highest pH value of 4.39 while the control was 4.89. It can be explained due to the increasing amount of probiotic bacteria which is decreasing the pH values. This result seems to be consistent with other researchers which found the pH values of 4.45 after a storage time of 7 days for kefir synbiotic drink.¹⁸ The Tukey test for the pH level showed that all the kefir drinks with the addition of inulin were significantly different from the control.

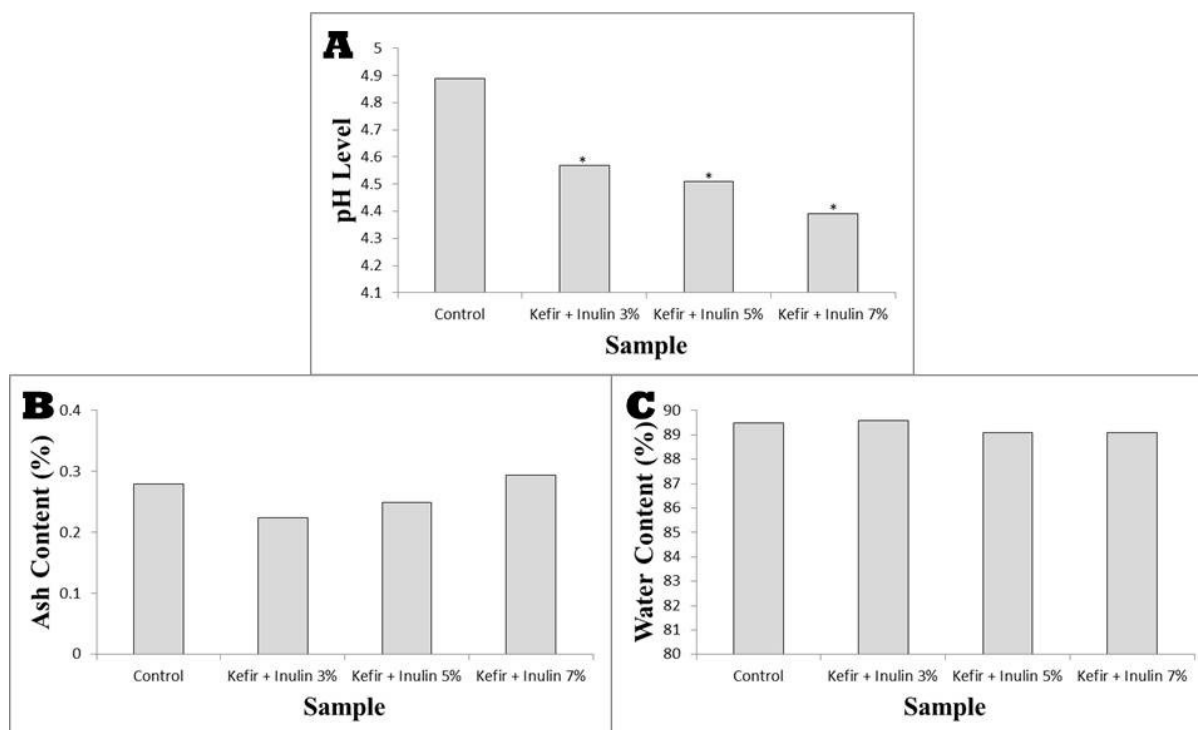


Fig.-5: The Physicochemical Properties of Kefir Synbiotic Drink: (A) pH Level; (B) ash Content; and (C) Water Content.

For the water and ash content of kefir synbiotic drink, the addition of inulin does not have a significant effect based on the Tukey test ($p < 0.05$). The water content of all synbiotic drink samples remains similar to the control with values ranging from 89.1 – 89.5% (Fig.-5B). The ash content showed value in a range of 0.224 – 0.294% as can be shown in Fig.-5C.

Biological Properties

The fat content of the synbiotic drink was determined using the AOAC method and the result was presented in Fig.-6A. It can be seen that the fat content of kefir synbiotic drink has a similar result with the control. The fat contents are range from 0.23 – 0.24%. This finding is in association with the previous result which also found the fat content of kefir synbiotic drink from skim milk powder which contained 0.2% fat.

The protein content of kefir synbiotic drink was measured using the Kjeldahl method. Figure-6B depicts the protein content of all samples. It is apparent from that Fig.-6B that the protein contents were decreased in addition to inulin. The protein content decreased 0.61% from the control to 3.54% for kefir enriched inulin 7%. The Tukey test of different means of all kefir synbiotic drinks compared to control showed the significant effect of protein content.

Lactic acid is the major product of kefir-inulin synbiotic drink fermentation and provides a sour taste on the product.¹⁹ From the graph, in Fig.-6C the lactic acid content was increased in the increasing inulin concentration. This is presumably due to inulin providing a good medium for lactic acid bacteria to grow.²⁰ Other researchers found that the lactic acid concentration was increased after 7 days of storage time compared to one day on non-fat kefir enriched with inulin.¹⁹

Sensory Analysis

Figure-7 shows the radar plot graph of sensory evaluation of kefir synbiotic drink. The panel group containing 25 untrained people was asked their perception of the aroma (Fig.-7A) and taste (Fig.-7B) of the synbiotic drink. The Tukey test showed significant ($p < 0.05$) differences in the aroma for kefir enriched inulin 3 and 5%. In contrast, there is no significant differences were confirmed by the Tukey test for taste assessment. What is surprising is that the previous researcher has reported that addition inulin on

kefir drink has worse taste than the control.²⁰ However this present result consistent with previous study which concluded that addition inulin on kefir synbiotic drink did not have a significant effect on taste score based on panelist perception.¹⁹

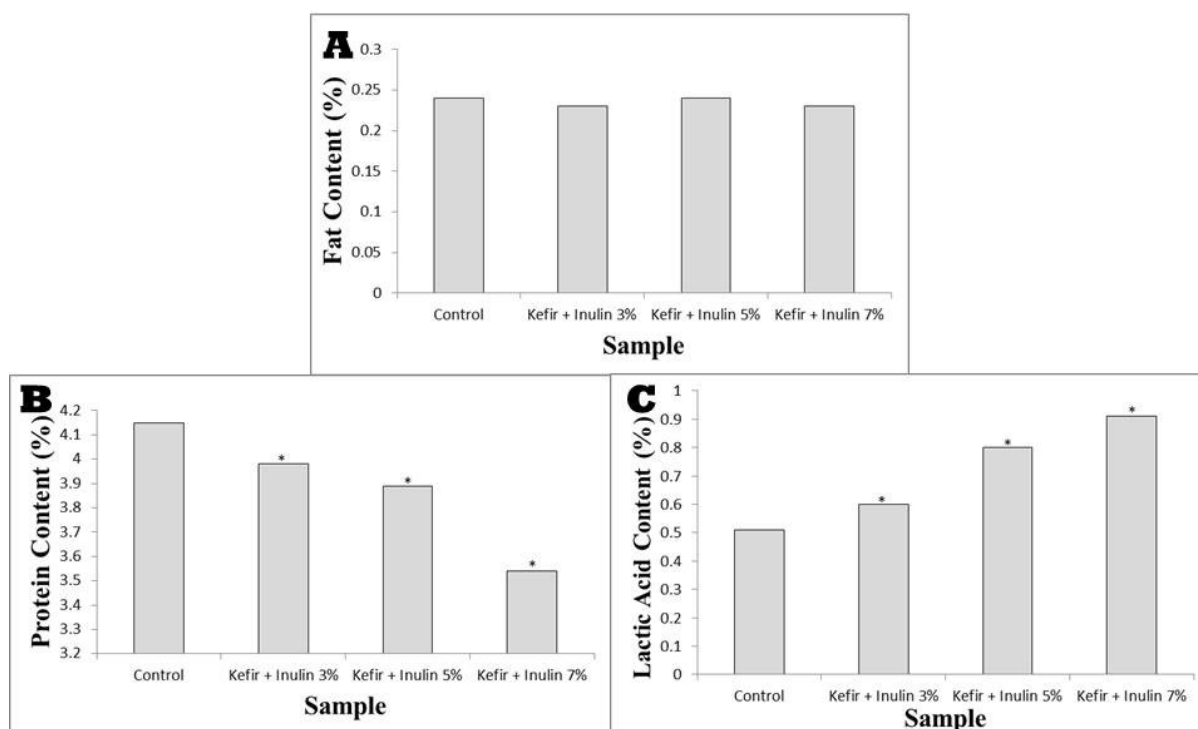


Fig.-6: The Biological Properties of Kefir Synbiotic Drink: (A) Fat Content; (B) Protein Content; and (C) Lactic Acid Content.

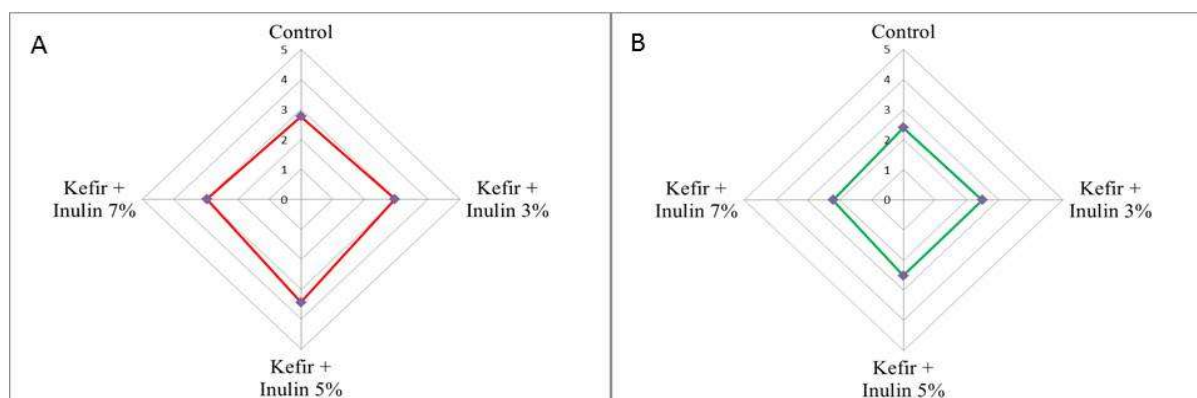


Fig.-7: The Radar Plot Graph of Sensory Analysis for (A) Aroma and (B) Taste.

CONCLUSION

This research showed that inulin can be extracted from dahlia tuber with a yield of 1.86% based on its fresh weight. The FT-IR spectrum of extracted and commercial inulin depicted almost similar. The addition of inulin to kefir drinks has increased the gram-positive bacteria as affirmed by the crystal-violet iodine complex test. The kefir synbiotic drink performed a good antimicrobial activity and the colony unit bacteria calculated was higher than control and standard. The physicochemical and biological properties showed that the synbiotic drink has better properties than the control. It is worthy to note that inulin provides a good medium to growth for probiotic bacteria affirmed with increasing lactic acid content. The quality of synbiotic drink assessed by panelist showed that the kefir enriched inulin concentration of 3 and 5% have a significant difference in aroma determined by the Tukey test while no significant difference in taste between control and synbiotic drink obtained.

ACKNOWLEDGMENT

The authors wish to thank Universitas Sumatera Utara for using facilities in the Biochemical Laboratory – Department of Chemistry, Faculty of Mathematics and Natural Sciences.

REFERENCES

1. D. Mohanty, S. Misra, S. Mohapatra, and P. S. Sahu, *Food Bioscience*, **26**, 152(2018), <http://dx.doi.org/10.1016/j.fbio.2018.10.008>
2. M. Nazir, S. Arif, R. S. Khan, W. Nazir, N. Khalid, and S. Maqsood, *Trends in Food Science & Technology*, **88**, 513(2019), <http://dx.doi.org/10.1016/j.tifs.2019.04.011>
3. O. Kareb and M. Aider, *Probiotics and Antimicrobial Proteins*, **11**, 348(2019), <http://dx.doi.org/10.1007/s12602-018-9427-6>
4. I. Purwaningsih, R. Hardiyati, M. Zulhamdani, C. S. Laksani, and Y. Rianto, *Jurnal Teknologi dan Industri Pangan*, **32**, 83(2021), <http://dx.doi.org/10.6066/jtip.2021.32.1.83>
5. Q. Ye, N. Georges, and C. Selomulya, *Trends in Food Science & Technology*, **78**, 167(2018), <http://dx.doi.org/10.1016/j.tifs.2018.05.025>
6. N. Kusumawati, A. Bahar, P. Setiarso, S. Muslim, and A. R. S. Auliya, *Rasayan J. Chem*, **14**, 1920(2021), <http://doi.org/10.31788/RJC.2021.1436331>
7. P. E. S. Munekata, J. Á. Pérez-Álvarez, M. Pateiro, M. Viuda-Matos, J. Fernández-López, and J. M. Lorenzo, *Trends in Food Science & Technology*, **113**, 397(2021), <https://doi.org/10.1016/j.tifs.2021.05.025>
8. B. Esmaeilnejad Moghadam, F. Keivaninahr, M. Fouladi, R. Rezaei Mokarram, and A. Nazemi, *International Journal of Dairy Technology*, **72**, 183(2019), <http://dx.doi.org/10.1111/1471-0307.12579>
9. P. Glibowski and K. Skrzypczak, Chapter 6 - Prebiotic and Synbiotic Foods, In: Holban, AM and AM Grumezescu (Eds), *Microbial Production of Food Ingredients and Additives*, Academic Press, 2017, pp.155-188, <http://dx.doi.org/10.1016/B978-0-12-811520-6.00006-4>
10. C. Kumalasari, M. Muchtaridi, I. Setiawan, and L. Adriani, *Rasayan J. Chem*, **13**, 1719(2020), <http://dx.doi.org/10.31788/RJC.2020.1335764>
11. M. Faisal, Saifullah, and T. Mukhriza, *Rasayan J. Chem*, **12**, 1151(2019), <http://dx.doi.org/10.31788/RJC.2019.1235365>
12. N. Gupta, A. K. Jangid, D. Pooja, and H. Kulhari, *International Journal of Biological Macromolecules*, **132**, 852(2019), <http://dx.doi.org/10.1016/j.ijbiomac.2019.03.188>
13. G. Tripodo and D. Mandracchia, *European Journal of Pharmaceutics and Biopharmaceutics*, **141**, 21(2019), <http://dx.doi.org/10.1016/j.ejpb.2019.05.011>
14. R. S. Singh, T. Singh, and C. Larroche, *Bioresource Technology*, **273**, 641(2019), <http://dx.doi.org/10.1016/j.biortech.2018.11.031>
15. W. Kosasih, S. Pudjiraharti, D. Ratnaningrum, and S. Priatni, *Procedia Chemistry*, **16**, 190(2015), <http://dx.doi.org/10.1016/j.proche.2015.12.035>
16. B. H. Çadirci and S. Çitak, *Pakistan Journal of Nutrition*, **4**, 237(2005), <http://dx.doi.org/10.3923/pjn.2005.237.241>
17. O. T. Ranzani, A. Motos, C. Chiurazzi, A. Ceccato, M. Rinaudo, G. Li Bassi, M. Ferrer, and A. Torres, *Clinical Microbiology and Infection*, **26**, 1456(2020), <https://doi.org/10.1016/j.cmi.2020.08.015>
18. P. Glibowski and E. Zielińska, *International Journal of Dairy Technology*, **68**, 602(2015), <http://dx.doi.org/10.1111/1471-0307.12234>
19. B. Ertekin and Z. B. Guzel-Seydim, *Journal of the Science of Food and Agriculture*, **90**, 543(2010), <http://dx.doi.org/10.1002/jsfa.3855>
20. L. Tratnik, R. Bozanic, Z. Herceg, and I. Drgalic, *International Journal of Dairy Technology*, **59**, 40(2006), <http://dx.doi.org/10.1111/j.1471-0307.2006.00236.x>

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