



Microbiological characteristics of goat's milk kefir with the addition of mangosteen (*Garcinia mangostana L.*) peel extract

Listya Purnamasari^{1*}, Defi S. Cahyaningrum², Mochammad S. Sunarno², Viagian Pastawan³, and Epi Taufik²

¹ Department of Animal Husbandary, Faculty of Agriculture, University of Jember, Indonesia

² Department of Animal Production and Technology, Faculty of Animal Science IPB University, Indonesia

³ Faculty of Applied Biological Sciences Gifu University, Japan

Abstract

Kefir is one of the fermented milk products. The addition of mangosteen peel extract, as a source of antioxidant, was aimed to increase the functionality of fermented milk products. The purpose of this research was to study the microbiological characteristics of goat's milk kefir with the addition of different concentration (0%, 1%, 2%, and 3%) of mangosteen peel extract. The extract of mangosteen peel was purchased from PT. Haldin Pacific Semesta. The results showed that the addition of mangosteen peel extract did not affect significantly in total lactic bacteria, total plate count, and total coliform; but it affected significantly in total mold and yeast. Total lactic acid bacteria in kefir decreased from kefir grain and bulk starter of kefir, but those populations are still same with the standard of microorganisms in prebiotic, which is 10^7 CFU/ml. The conclusion of this study, mangosteen peel extract can be used to decrease the total microbial such as mold/yeast at concentration 3% and 4%.

Article History

Received August 21, 2020

Accepted December 16 2020

Keyword

Goat's milk kefir, Mangosteen peel extract, Microbiological activity.

1. Introduction

Public awareness of the importance of health is increasing. This is indicated by the development of functional food products that produced by the food industry in the middle of society. Food functional according to Wildman (2001) is food and food ingredients can provide some benefits besides the basic nutritional function of the food in the society (1). One of the functional food products, which is currently being developed by the milk processing industry, is kefir. Kefir belongs to one type of functional food because kefir is not only useful as a source of nutrients, but also the non-nutritional bioactive components that are very strategic to maintain the health and fitness of the human body (2). Kefir is a fermented milk product with pure culture kefir seeds. Kefir seeds are a set of lactic acid bacteria (BAL) such as; *Lactobacilli*, *Streptococcus sp*, and several types of non-pathogenic yeast/mold. The fermentation process is carried out by microorganisms that found in kefir seeds then breakdown the contents of milk as a basic ingredient for making kefir. The fermentation process produces lactic acid as a result of metabolism Lactic acid bacteria (LAB), which are antimicrobial against pathogenic bacteria, because it inhibits the growth of pathogenic bacteria by lowering the pH.

Mangosteen peel extract is one of the herbal extracts that currently began to be widely used by the public and the pharmaceutical industry, because it has many health

* Correspondence : Listya Purnamasari  listyap.faperta@unej.ac.id

benefits. Mangosteen peel extract in Indonesia is obtained easily. This is because the mangosteen plants come from tropical forests in the Southeast Asian region, such as the jungles of Indonesia and Malaysia, so the amount of mangosteen in Indonesia is abundant. The abundant amount of mangosteen fruit in Indonesia is characterized by increased exports of mangosteen fruit to Singapore, Hongkong, and England. In 1999, the export volume of mangosteen fruit reached 3887816 US \$ and in 2000 the export volume reached 7182098 kg with an export value of 5885038 US \$ (ICUC 2003). Benefits of mangosteen peel extract for health has proven by Chairungsrikerd (1996) study of antihistamine ability in the aortic contraction of the isolated rabbit's chest induced by histamine or serotonin, the study of Moongkarndi *et al.* (2004) about mangosteen peel extract shows the potential activity in inhibiting breast cancer cell proliferation and showing apoptotic activity, and other studies that examine benefits the pharmacology of mangosteen peel extract (3,4).

Mangosteen peel extract also has a strong antimicrobial activity derived from xanthone compounds, so the addition of mangosteen peel extract on kefir is believed to increase the benefits of kefir. Antimicrobial activity in mangosteen peel extracts proved by the research of Suksamrarn *et al.* (2003) (5) concerning xanthone compounds in mangosteen peel extract which shows strong inhibitory activity against tuberculosis microbe. Antimicrobial activity on mangosteen peel extract also has possibilities that affect the number of microorganism populations in kefir, so microbiological characteristics were tested on goat milk kefir that added by mangosteen peel extract. The purpose of this study is to examine the microbiological characteristics in goat milk kefir added by commercial mangosteen peel extract with different concentrations as a functional food.

2. Materials and Methods

This research involved of making a bulk starter, making kefir, determining the microbiological quality of kefir grain, kefir bulk starter, skim goat milk, mangosteen peel extract, and kefir microbiological analysis. Variables observed of microbiological characteristics are the total plate count population, lactic acid bacteria, mold, yeast, and coliform contamination.

2.1. Making Kefir Bulk Starter (Burton, 2013)

Goat's milk was separated from its fat (skim milk) as much as 2 liters pasteurized at 80°C for \pm 30 minutes then the temperature was reduced to 30°C. Kefir grains or pure culture were inoculated into milk as much as 5% (w/v), then incubated at room temperature \pm 28°C for 48 hours to become a bulk starter.

2.2. Kefir Bulk Starter Viability Check

Starter culture viability check was aimed to determine the amount of the colony in the starter which determined by fertilizing, and kefir bulk starter was grown on MRSA media. The fertilization method refers to the National Standardization Agency (2009) (6).

2.3. Making Kefir (Burton, 2013)

Goat's skim milk was obtained from fresh separated goat milk from the fat with a cream separator. The obtained skim milk was heated until reached the temperature of 85°C for \pm 30 minutes. Goat's skim milk was cooled to room temperature ($28 \pm 1^\circ$ C). Bulk Starter was inoculated as much as 10% of the volume of goat skim milk and then added the mangosteen peel extract as much as 0%, 1%, 2%, and 3% as a treatments. Kefir, which has

been homogenized, was then incubated at room temperature for 48 hours. The diagram how to make goat's milk kefir with mangosteen peel extract can be seen in Figure 1.

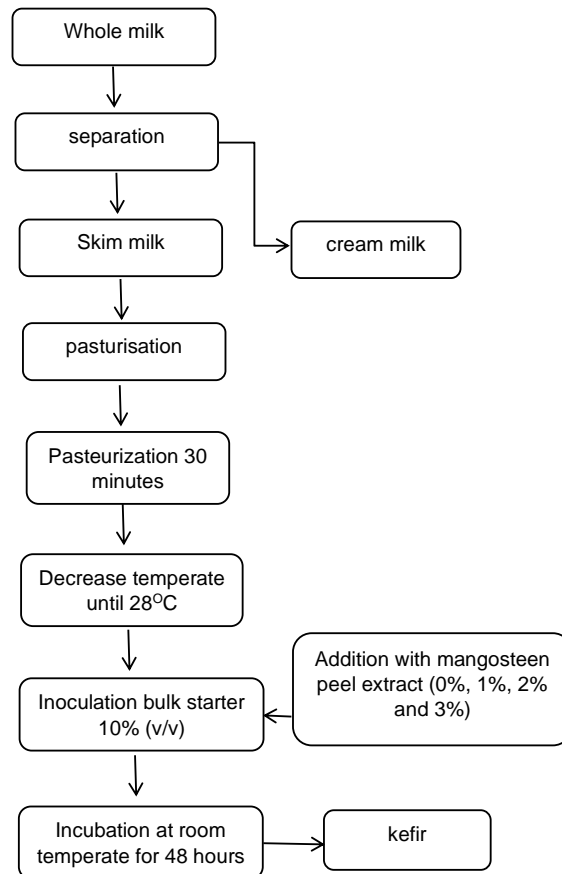


Figure 1 Diagram for making kefir by adding mangosteen peel extract

2.4. Testing the Kefir Microbiological Characteristics

Method of testing the microbiological characteristics of goat milk kefir is using the pouring method (7). One milliliter of the sample obtained from the desired dilution was fertilized into a sterile petridisk. Next, 12-15 ml of media were added to the each media on petri dish. Homogenization was done by moving petridisk form the number eight. After the agar cup freezes, the cup was incubated upside down. The number of microorganisms, that are counted, is the number of lactic acid bacteria, total mold, yeast, total of all colonies, and coliforms. Microbial Colony was calculated based on the standard plate count refered to BAM (2011) with the following formula (8):

Total colonies

$$\left(\frac{CFU}{ml} \right) \frac{(n1+n2)}{2} \times N^{-1}$$

Information:

n1= total colony first petridisk

n2= total colony second petridisk

N=dilution factor

2.5. Data Analysis

The randomized group design (RBD) was used in this study to evaluate microbiological characteristics of goat's milk kefir with the addition of commercial mangosteen peel extract at a different concentration (0%, 1%, 2%, and 3%). The variables observed in this study were microbiological characteristics kefir including total population of coliforms, lactic acid bacteria, molds and yeasts, and TPC. Data analysis. Microbiological quality data obtained from this study were tested assumption with Minitab 14 software then if the results of the assumption test meet the variance test using statistical analysis with analysis of variance (ANOVA) using SAS software 9.1.3, then continued with Duncan's test

3. Results and Discussion

3.1. Microbiological Characteristics of Goat Milk

Testing the quality of goat milk is done before milk is used as raw material for making kefir. The quality of raw materials is important because it will affect the quality of the fermentation results. Table 1 shows the quality of microbiological raw materials for milk used.

Table 1. Microbiological characteristic of goat's skim milk

Variable	Result (log ₁₀ CFU/ ml)
Coliform	<1.0
Total Plate Count	5.18
Mold/yeast	<1.0
Lactic Acid Bacteria	5.60

This study was using fresh goat's milk that has been desperated to remove milk fat that called skim goat's milk. Microbiological analysis results showed no growth of coliform and yeast/mold on skim goat's milk (<1.0 log₁₀ CFU/ ml). Analysis results showed that the amount of TPC of 5.18 log₁₀ CFU/ ml qualified to the requirements established by Thai Agricultural Standard Raw Goat Milk (2008) (9). Thai Agricultural Standard Raw Goat Milk (2008) said that goat milk has several types of quality based on the number of TPC. Goat milk with premium quality has a TPC of less than 5.0×10^4 CFU/ ml, goat milk with good quality has a TPC 5.0×10^4 CFU/ ml and goat milk with quality standards have TPC 10^5 to 2.0×10^5 CFU/ ml, so The goat's milk that used in this study is included in the quality standard.

Raw goat milk in the United States according to Zweifel *et al.* (2005) (10), had a total bacterial allowance of a maximum of 5.0 log₁₀ CFU/ ml with a number of microorganisms 6.0 log₁₀ CFU/ ml. Frequent microorganisms used as an indicator of sanitation in food products is coliform bacteria. The presence of coliform in food indicates that contamination has occurred on products (11). Goat skim milk as raw material for making kefir has coliform contamination of less than 1.0 log₁₀ CFU/ ml. Contaminant amount of the coliform is still in the standard amount of coliform contamination in goat milk based on Thai Agricultural Standard Raw Goat Milk (2008), which is less than 3.0 log₁₀ CFU/ ml. The amount of mold and yeast in goat milk is less than 1 log₁₀ CFU/ ml. The number of mold and yeast is below the number of molds and yeast of goat milk that has been studied by Suguna *et al.* (2012)(12), which is 4.2 - 4.6 log₁₀ CFU/ ml. The used amount of coliform, mold, and yeast

of the goat milk is still in the standard range. This matter shows that the process of milking and handling goat's milk in animal husbandry is according to GHP (Good Handling Practices)

3.2. Microbiological Characteristics of Kefir Grain and Kefir Bulk Starters

Microbiological analysis of kefir grain and bulk starter of kefir is importantly conducted to determine the quality and eligibility as a starter making kefir. Table 2 shows the microbiological quality of kefir grains and bulk starters kefir.

Table 2. Microbiological characteristic of kefir grains and bulk starters kefir

Variable	Result (\log_{10} CFU/ ml)	
	kefir grains	bulk starters kefir
Coliform	<1.0	<1.0
Total Plate Count	7.60	8.06
Mold/yeast	4.60	4.60
Lactic Acid Bacteria	7.60	7.60

Coliform bacteria is one of the pathogenic bacteria in the food product preparations derived from the family Enterobacteriaceae which have flagella monotrichous. This group of bacteria is often used as an indicator of their presence contaminants in food products. Test results on kefir grain as raw material for making kefir bulk starter in this study have contamination coliform less than $1.0 \log_{10}$ CFU / ml. The population of coliform bacteria contamination in Kefir bulk starter obtained from the test is also less than $1.0 \log_{10}$ CFU/ ml. These results indicate that the amount of coliform population contamination the kefir grain and bulk starter kefir is still in limit standards of coliform contamination on fermented milk according to SNI 7388-2009, fermented milk has a maximum limit of coliform contamination is $1.0 \log_{10}$ CFU/ ml.

The total number of microbial (TPC) kefir grains and bulk starter kefir in this study, which was calculated by the SPC method, was $7.60 \log_{10}$ each CFU/ ml and $8.06 \log_{10}$ CFU/ ml. The results of the calculation of the total number of microbial kefir grains and kefir bulk starter have the minimum TPC kefir starter on CODEX 243-2003, the minimum TPC is 1.0×10^7 CFU/ g.

In contrast to other types of fermented milk products, kefir, and koumiss has a standard number of mold and yeast. Based on previous research showed that mold and yeast in kefir have the amount which is less than kefir bacteria, although mold and yeast in kefir have a clear role which is to provide a good environment for kefir bacterial growth and produce influential metabolites flavor and mouthfeel from kefir production. The results of the calculation of the number of mold and yeast on kefir grains are $4.60 \log_{10}$ CFU/ ml and bulk kefir starter of $4.60 \log_{10}$ CFU/ ml meets the CODEX 243-2003 standard mention that the minimum amount of mold and yeast in kefir products is 1.0×10^4 CFU/ g. Calculation of the number of lactic acid bacteria (BAL) in kefir grain and bulk kefir starter in this study were same, $7.60 \log_{10}$ CFU/ ml. The total LAB content in the kefir grain and bulk starter of the kefir by the number of BAL based on Farnworth (2005) which shows kefir grain and kefir bulk starter have a BAL number of $7.37-8.94 \log_{10}$ CFU/ g and $7.60 - 8.43 \log_{10}$ CFU/ g, respectively.

3.3. Microbiological Characteristics of Mangosteen peel extract

The raw material added in this study is a mangosteen peel extract. Table 3 shows the extra microbiological characteristics of mangosteen peel.

Table 3. Microbiological characteristic of kefir grains and bulk starters kefir

Variable	Result (\log_{10} CFU/ ml)	
	PT Haldin Pacific Semesta	SNI 7388-2009
Coliform	<1.0	<2.0
Total Plate Count	<1.0	6.00
Mold/yeast	<1.0	4.30
Lactic Acid Bacteria	<1.0	-

The observation of microbiological characteristics of mangosteen peel extract on coliform variables, TPC, yeast molds, and LAB showed no detection of its growth. Based on the results of the analysis that has been done, the extract mangosteen peel obtained from the manufacturer by National Standards Indonesia 7388-2009 concerning the maximum limit of microbial contamination in food for herbs and spices that has a TPC number of less than 6 \log_{10} CFU/ g and mold and yeast less 4.3 \log_{10} CFU/ g (Indonesian National Standard 2009) so that it can be used as raw material for making kefir (6).

3.4. Microbiological Characteristics of Goat's milk kefir addition with commercial mangosteen peel extract

The microbiological characteristics analysis of goat milk kefir aims to know the microbiological characteristics of the final product so that it can be known safety and suitability of the consumed product. Microbiological characteristics of goat's milk kefir products were observed of total microorganisms (TPC), lactic acid bacteria, yeast, and coliform. Microbiological characteristics of milk kefir goat with the treatment of adding different mangosteen peel extracts presented in Table 4.

Table 4. Microbiological characteristic of goat's milk kefir addition with different concentration of mangosteen peel extract

Variable	Result of the treatment (\log_{10} CFU/ ml)			
	P1	P2	P3	P4
Coliform	<1.0	<1.0	<1.0	<1.0
Total Plate Count	8.36 \pm 1.32	8.20 \pm 1.04	9.01 \pm 2.43	8.89 \pm 1.14
Mold/yeast	6.29 \pm 0.27a	7.80 \pm 0.20b	7.03 \pm 0.55ab	6.67 \pm 0.88ab
Lactic Acid Bacteria	6.07 \pm 0.92	7.53 \pm 0.41	7.20 \pm 0.80	7.14 \pm 0.96

abc = Significant at 5% level of significance of mangosteen peel extract concentration; P1 = Goat's milk kefir with 0% mangosteen peel extract; P2 = Goat's milk kefir with 1% mangosteen peel extract; P3 = Goat's milk kefir with 2% mangosteen peel extract; P4 = Goat's milk kefir with 3% mangosteen peel extract

The results of microbiological characteristics analysis of goat's milk kefir products with the addition of mangosteen peel extract 0%, 1%, 2%, and 3% showed that there were no

significant differences in the observed variables, total coliform, total plate count and lactic acid bacteria of goat's milk kefir product with the addition of mangosteen peel extract 0% - 3% which had coliform contamination of less than $1 \log_{10}$ CFU/ ml. The amount of coliform in the kefir regarding to SNI 7388-2009, limits maximum coliform contamination in fermented milk, is less than $1.0 \log_{10}$ CFU/ ml. Kefir is a product that produced from fresh milk and will be consumed directly by consumers, so coliform contamination must be below the standards. The amount of coliform in this kefir product is appropriate to the SNI standards. Its indicated that sanitation and hygiene during handling the process of making kefir was guaranteed well. TPC in goat milk kefir products with the addition of mangosteen peel extract 0% -3% shows no difference which has range from 8.20- 9.01 \log_{10} CFU/ ml. TPC in goat's milk kefir according to the standard CODEX STAN 243-2003, minimal TPC in kefir, is $7 \log_{10}$ CFU/ ml. The amount of LAB in goat's milk kefir with addition mangosteen peel extract 0% -3% also showed no difference 6.07-7.53 \log_{10} CFU/ ml. Amount of LAB and other microorganisms in this kefir according to the CODEX STAN 243-2003, kefir is ready to drink so the microorganism content at least $7 \log_{10}$ CFU/ ml. The number of LAB in kefir decrease from the number of LAB in the bulk starter. The decrease in the number of LAB in kefir occurs due to the treatment addition of mangosteen peel extract which can inhibit bacterial growth. Maliana et al. (2013) (13) explained that mangosteen peel contains chemical compounds as antibacterial. Bioactive compounds contained in mangosteen peel extract comes from the flavonoid group, alkaloids, terpenoids, polyphenols, quinones, and tannins. Decreased lactic acid bacteria in kefir still meets the minimum total amount of bacteria in kefir according to CODEX STAN 243-2003 which is a minimum of 10^7 CFU/ g.

The results of the analysis data on the number of molds and yeasts in goat's milk kefir without the addition of mangosteen peel extract shows the significant difference ($P < 0.05$) compared to the number of mold and yeast kefir of goat milk with mangosteen peel extract. Kefir without the addition of mangosteen peel extract has a number of molds and yeasts ($6.29 \pm 0.27 \log_{10}$ CFU/ ml) which markedly lower than kefir that added by mangosteen peel extract 1%, 2% and 3% ($7.80 \pm 0.20 \log_{10}$ CFU/ ml, $6.76 \pm 0.88 \log_{10}$ CFU/ ml, $7.03 \pm 0.55 \log_{10}$ CFU/ ml). These results indicate that the addition of mangosteen peel extract in kefir can increase the number of mold and yeast in kefir. The amount of mold and yeast in kefir with the addition of mangosteen peel extract at different levels is higher than kefir without additional mangosteen peel extract, but the greater levels of the addition of mangosteen peel extract, the amount of mold and yeast in kefir also decreases.

The amount of mold and yeast in kefir with mangosteen peel extract is higher because the addition of mangosteen peel extract can increase the number of carbohydrates in kefir. Carbohydrates are the best source of energy for yeast metabolism in the process of yeast growth in its environment (14). Carbohydrate content in skin extracts mangosteen from PT. Haldin Pacific Universe amounted to 93.87% of the total its chemical composition is due to a mixture of starch (Burton 2013). Decrease the amount of mold and yeast in kefir along with the addition of extract levels mangosteen peel also occurred due to an increase in antimicrobial activity on kefir. Putra (2010) (15) described the antimicrobial compounds contained in mangosteen peel extract comes from phenol compounds, terpenes, alkaloids, and polypeptides. Putra (2010) in his research stated that the identification of fraction compounds ethyl acetate methanol extract of mangosteen rind by chromatography method shows that the dominant component is xanthone by 38.92% (15). The xanthone compound on mangosteen rind has antimicrobial activity against mold (16). This causes the higher

addition of mangosteen peel extract then the amount of mold and yeast on kefir will decrease further. Kefir products with the addition of mangosteen peel extract produced in this study is still feasible to declare probiotic drinks. This is because these kefir products as the probiotic requirements because the population of lactic acid bacteria is more than $7 \log_{10}$ CFU/ ml.

4. Conclusions

The addition of mangosteen peel extract into goat's milk kefir with a different concentration did not affect the number of TPC, LAB, and coliform contamination, however, the addition of mangosteen peel extract affected the amount of mold and yeast in kefir. Viability of lactic acid bacteria in kefir with the addition of mangosteen peel extract decreased from kefir grain and bulk kefir starter, but the population still meets the minimum amount requirements total microorganism in kefir is $7 \log_{10}$ CFU/ ml.

Acknowledgements

All sources of funding of the study should be disclosed. Please clearly indicate grants that you have received in support of your research work. Clearly state if you received funds for covering the costs to publish in open access.

Author Contributions

Defi Syukria Cahyaningrum, Epi Taufik and M. Sriduresta Sunarno conceived and designed the experiments; Listya Purnamasari and Defi Syukria Cahyaningrum performed the experiments and analyzed the data; Epi Taufik and M. Sriduresta contributed reagents/materials/analysis tools; Listya Purnamasari and Defi Syukria Cahyaningrum wrote the paper.

References

1. Wildman REC. Handbook of Functional Food and Nutraceuticals. Boca Raton: CRC Press; 2001.
2. Wijaningsih W. Aktivitas Antibakteri In Vitro Dan Sifat Kimia Kefir Susu Kacang Hijau (*Vigna Radiata*) Oleh Pengaruh Jumlah Starter Dan Lama Fermentasi In Vitro Antibacterial Activity And Chemicalproperties Of Mungbean Milk Kefir (*Vigna Radiata*) As Affected By Cultures Concentration Andfermentation Time. Program Pasca Sarjana Universitas Diponegoro; 2008.
3. Chairungsrilerd N, Furukawa K-I, Ohta T, Nozoe S, Ohizumi Y. Pharmacological properties of α -mangostin, a novel histamine H1 receptor antagonist. *Eur J Pharmacol.* 1996;314(3):351–6.
4. Moongkarndi P, Kosem N, Kaslungka S, Luanratana O, Pongpan N, Neungton N. Antiproliferation, antioxidation and induction of apoptosis by *Garcinia mangostana* (mangosteen) on SKBR3 human breast cancer cell line. *J Ethnopharmacol.* 2004;90(1):161–6.
5. Suksamrarn S, Suwannapoch N, Phakhodee W, Thanuhiranlert J, Ratananukul P, Chimnoi N, et al. Antimycobacterial activity of prenylated xanthenes from the fruits of *Garcinia mangostana*. *Chem Pharm Bull.* 2003;51(7):857–9.
6. Indonesia SN. Batas maksimum cemaran mikroba dalam pangan. SNI. 2009;7388:2009.
7. Fardiaz DS. Mikrobiologi pangan 1. PT Gramedia; 1992.
8. Manual BA. Aerobic plate count. US Food Drugs Adm. 2001;
9. Standard TA. Raw goat milk. Natl Bur Agric Commod Food Stand Minist Agric Coop Thail. 2008;

10. Zweifel C, Muehlherr JE, Ring M, Stephan R. Influence of different factors in milk production on standard plate count of raw small ruminant's bulk-tank milk in Switzerland. *Small Rumin Res.* 2005;58(1):63–70.
11. M S. Analisis koliform dalam susu segar sebagai parameter sanitasi peternakan. Institut pertanian Bogor; 2008.
12. Suguna M, Bhat R, Nadiah WAW. Microbiological quality evaluation of goat milk collected from small-scale dairy farms in Penang Island, Malaysia. *Int Food Res J.* 2012;19(3):1241.
13. Maliana Y, Khotimah S, Diba F. Aktivitas antibakteri kulit *Garcinia mangostana* Linn. terhadap pertumbuhan *Flavobacterium* dan *Enterobacter* dari *Coptotermes curvignathus* Holmgren. *Protobiont.* 2013;2(1).
14. Rodrigues F, Ludovico P, Leão C. Sugar metabolism in yeasts: an overview of aerobic and anaerobic glucose catabolism. In: *Biodiversity and ecophysiology of yeasts.* Springer; 2006. p. 101–21.
15. Putra INK. Aktivitas antibakteri ekstrak kulit buah manggis (*Garcinia mangostana* L.) Serta kandungan senyawa aktifnya [Antibacterial Activity of Mangosteen (*Garcinia mangostana* L.) Husk Extract, and its Active Compounds]. *J Teknol dan Ind Pangan.* 2010;21(1):1.
16. Gopalakrishnan G, Banumathi B, Suresh G. Evaluation of the antifungal activity of natural xanthenes from *Garcinia mangostana* and their synthetic derivatives. *J Nat Prod.* 1997;60(5):519–24.