Livestock and Animal Research
Accredited by Directorate General of Higher Education, Research, and Technology No. 152/E/KPT/2023

Open Access
Livest. Anim. Res., March 2024, 22(1): 58-66
p-ISSN 2721-5326 e-ISSN 2721-7086
https://doi.org/10.20961/lar.v22i1.78614

Original Article

Characteristics of functional ice cream produced with goat's milk kefir in combination with mono-acylglycerol

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Received: September 7th, 2023; Accepted: January 30th, 2024; Published online: March 5th, 2024

Abstract

Objective: The purpose of this study was to determine the character of ice cream made from goat's milk kefir (GMK) combined with mono-acylglycerol (MAG).
Methods: GMK was produced by pasteurized goat's milk (85°C for 15 s) and 2% kefir grain. Ice cream mix (ICM) was produced by GMK, MAG (T0= 0%, T1= 0.25%; T2= 0.50%; T3= 0.75%), 4% sucrose, and 0.1% salt. The results of observations processed using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) if there were a significant difference between treatments.
Results: Es puter (EP) made from GMK in combination with MAG has a distinctive character against T0 (control). The use of GMK decreases the pH value, thereby increasing the lactic acid and ethanol content which brings out the acidity of the product ($p<0.05$). In addition, increasing MAG concentration can improve the EP's overrun to make the product more melt resistant ($p<0.05$). The use of GMK combined with MAG can support the viability of lactic acid bacteria (LAB) and yeast through storage in the freezer. The number of viable LAB and yeast meets WHO standards to obtained health benefit from consuming probiotic ice cream.
Conclusions: To conclude, EP made from GMK in combination with 0.50% MAG was proved to be the best treatment.

Keywords: Acidity; Melting rate; Overrun; Probiotic ice cream; Viability

INTRODUCTION

Kefir is a food derived from processed cow's milk [1–4], buffalo's milk [5–7], and goat's milk [8,9]. So far, the study that have been carried out about kefir products have been limited. Latest investigations on kefir ice cream were physicochemical and sensorial quality [9]. Meanwhile, the latest study on probiotic ice cream still does not use kefir as research material [10,11]. Goat's milk kefir (GMK) has potential as a probiotic-based functional food because it consists of lactic acid bacteria (LAB) and yeast.

The addition of raw materials rich in fat and sugar is now starting to be replaced by using low-fat and low-sugar raw materials. Sulmiyati *et al.* [12] explained that the use of low-fat and low-sugar raw materials has a significant influence on the sensory quality like affecting the taste, texture, and acceptance of the product. According to Zoumpopoulou *et al.* [13], the utilization of *Limosilactobacillus fermentum* ACA-DC 179 to exert potential probiotic effects of ice cream. Kozłowicz *et al.* [15] state that the addition of probiotics supplemented with zinc does not affect the sensory quality of ice cream. According to Singh *et al.* [16], the use of probiotic to produce fermented milk-based ice cream successfully has a probiotic viability amount above 10^6 CFU/g and does not affect physicochemical quality. Probiotic combination can be found naturally in kefir. Kefir contains at least 10^6 and 10^4 CFU/mL of probiotics consisting of LAB and yeast respectively [3].

The use of GMK as a raw material for milk substitutes certainly affects the quality of ice cream. Qin *et al.* [2] explain that the fat content of kefir was lower than milk. Fermentation carried out by kefir grains causes catabolic reactions in milk fat and sugar [3]. According to Dong *et al.* [4], fat content affects the texture and body of ice cream. Fat is able to trap air entering the mixture so that ice cream has an overrun with a certain value. Fats have ester compounds that serve as a source of flavor [5]. In addition, fat was also able to decrease the freezing point of ice cream [6]. The absence of fat in the mixture causes instability in the ice cream. Therefore, emulsifiers addition in to formula is important.

Mono-diacylglycerol is a type of emulsifier commonly used by the food industry as an emulsifying agent [7]. Radiati *et al.* [8] explained that as many as 70% of emulsifiers most often used by the food industry was mono-diacylglycerol because it has a Generally Recognize as Safe (GRAS) status so it is safe to use in the ice cream production process. The mono-diacylglycerol used in this study was a commercial emulsifier (Dawn Food Product Inc, U.K) containing 93.30% mono-acylglycerol (MAG) and 6.70% di-acylglycerol. Mono-diacylglycerol in this study will henceforth be referred to as MAG.

MATERIALS AND METHODS

Preparation of GMK

The GMK in this study was produced based on protocol by Sulmiyati *et al.* [12] with minor modification. The goat's milk was obtained from Malang, East Java. Kefir grains (Brand: Kefiree) was purchased from Healtee Stuff Inc (Jakarta, Indonesia). The kefir grains identified were *Leuconostoc spp, Acetobacter spp, Klyuveromyces marxianus, Saccharomyces cerevisiae, S. unisporus, Lactobacillus paracasei, L. acidophillus, L. delbrueckii, L. plantarum, L. kefiranofaciens, L. kefiri,* and *Candida kefyr*. Kefir grains were propagated at 32°C for 1 day in the laboratory.

Preparation of EP

All EP in this study were produced based on protocol by Zoumpopoulou *et al.* [13]. All EP were prepared in accordance to achieve a total batch of 250 g ice cream. Ice cream mix (ICM) was prepared according to the following procedure. Sucrose, salt, and MAG as solid ingredients were weighed and mixed using a grinding bowl. Goat's milk as liquid ingredients were pasteurized up to 85°C for 15s in a stainless steel can. To achieve complete blending, solid ingredients were incorporated into the liquid ingredients to produce ICM. The ICM was pasteurized at 73°C for 10 min, cooled in an ice bath to reach 4°C, added with kefir grains and then aged at 4°C for 5 hours. Then, froze them using ice cream maker (GEA-1530, Indonesia) for 30 minutes. Finally, EP was packaged in 250 gr containers, sealed, and stored at -17°C.

Probiotic viability enumeration

Enumeration was carried out based on protocol by Goktas *et al.* [10] with slight modification. Sample (5 g) was homogenized with 2% sterile sodium citrate solution (45 ml, Sigma-Aldrich, USA). Probiotics were then enumerated as LAB and yeast. Yeast was incubated in yeast extract, peptone, dextrose (YPD) broth for 48 hours and LAB was incubated in de Man Rogosa Sharpe (MRS) broth for 24 hours at 37°C. NaCI (0.9%) solution serial dilution of EP was created and homogenized for 2 min. Serial dilutions were prepared and 100 μL from each dilution was spread to YPD and MRS agar plates for yeast and LAB, respectively. Probiotic viability was calculated using Equation 1.

$$\text{Probiotic Viability}=\frac{a}{((1\times b)+(0{,}1\times c)\times d)} \quad \ldots(1)$$

Where:
a = Calculated colony
b = Sum of Petri dish on the first dilution
c = Sum of petridish on the next dillution
d = First dilution

Characteristics determination of EP

Value of pH determination was carried out based on protocol by Indonesian National Standard 01-2891-1992 related to the National Standard Organization of food and beverage test method [14] with slight modifications. The acidity expressed as a percentage of lactic acid. The study sample was taken as much as 10 ml. Then put into Erlenmeyer glass. Then added 2 drops of Phenolphthalein indicator and titrated using NaOH 0.1 N until appear pink color. Acidity was calculated using Equation 2.

$$\text{Acidity}=\frac{a\times b\times c}{d\times 1000}\times 100\% \qquad \ldots\ldots(2)$$

Where
a = Volume of NaOH (ml)
b = Normality of NaOH
c = Molecul weight of lactic acid (90)
d = Mass of sample(g)

Overrun calculation was carried out based on protocol by Kozłowicz *et al.* [15] with slight modifications. Mass weighing carried out according to the theory of the equation that 1 gram was equivalent to 1 cm^3. Overrun was calculated using Equation 3.

$$\text{Overrun}=\frac{b-a}{a}\times 100\% \qquad \ldots\ldots(3)$$

Where:
a = Mass of ICM (g)
b = Volume of EP (cm^3)

The calculation of melting rate was carried out based on protocol by Sing *et al.* [16] with slight modifications. EP was conditioned to have a size of 6cm×6cm×3cm with a mass of ±100 g. The initial temperature of EP was -15°C. EP was placed on a stainless steel in a temperature-controlled chamber at 25°C. The melted volume of EP was recorded after 60 minutes of observation where 1 cm^3 equivalent to 1 gram and calculate using Equation 4.

$$\text{Melting rate}=\frac{a}{b}\times 100\% \qquad \ldots\ldots(4)$$

Where
a = Volume of melting EP (cm^3)
b = Mass of EP (g)

Observation of lactic acid levels was carried out based on protocol by Ouwehand *et al.* [17] with slight modifications. The percentage level of lactic acid determined by titration 0.1 N NaOH for 30 mL EP given 2 drops of phenolphthalein indicator until the color turn pink. Lactic acid was calculated using Equation 5.

$$\%\text{Lactic Acid}=\frac{a}{b}\times 100\% \qquad \ldots\ldots(5)$$

Where
a = Volume NaOH(mL)× $\frac{9}{1000}$
b = Mass of EP(gr)

Determination of ethanol contents was carried out based on protocol by Sarwar *et al.* [18] with slight modifications. 60 gr of EP was put into an erlenmeyer glass, dripped 2 drops of phenolphthalein indicator, and 300 mL of water. Ethanol contents was calculated using Equation 6.

$$\%\text{Ethanol}=\frac{a\times b\times c}{d}\times 100\% \qquad \ldots\ldots(6)$$

Where:
a = Means of titration result (mL)
b = Molarity of NaOH (0.1 N)
c = Mass relative of C_2H_5OH (46)
d = Mass of EP (g)

Data analysis

A randomized completely design with four concentrations of MAG (T0=0%; T1=0.25%; T2=0.50%; T3=0.75%) with three replications were designed to this study. The data were analyzed by analysis of variance (ANOVA) and if the effect was significant, the treatment differences were tested by Duncan's Multiple Range test (DMRT).

RESULTS

The treatment effect on the acidity, overrun, and melting rate of EP

The effect of using MAG in difference concentration in combination with GMK on acidity, overrun, and melting rate of EP were shown on Table 1. Combination MAG and GMK affects acidity, overrun, and melting rate of EP significantly. The results of DMRT tests on acidity and overrun observations show that significant differences only occur between T1 and T2. While in the melting rate observation, the DMRT test results show that a significant difference only occurs between control and T1.

Table 1. The effect of the combination of MAG and GMK on the acidity, overrun, and melting rate of EP

Character	Treatment			
	T0	T1	T2	T3
Acidity (%)	0.091^a±0.104	0.107^a±0.122	0.123^b±0.141	0.139^b±0.160
Overrun (%)	23.25^a±2.32	26.59^a±2.363	28.51^b±2.405	32.24^b±2.445
Melting rate (%)				
60 min	39^b±0.0588	34^a±0.0637	27^a±0.0647	25^a±0.0651

T0 = GMK + 0.00% MAG + 4% sucrose + 0.1% salt as control, T1 = GMK + 0.25% MAG + 4% sucrose + 0.1% salt, T2 = GMK + 0.50% MAG + 4% sucrose + 0.1% salt, T3 = GMK + 0.75% MAG + 4% sucrose + 0.1% salt, Numbers followed by different letters on the same line show a significant difference with a significance level of 5%.

The treatment effect on the pH value, lactic acid, and ethanol contents of EP

This study result show that the concentration of MAG in combination with GMK affects the pH value, lactic acid, and ethanol contents of EP ($p<0.05$). The pH value of EP decreases with an increase in MAG concentration. On the other hand, the content of lactic acid and ethanol contents increases as the concentration of MAG was increased combined with GMK to produce EP. The results of the DMRT test on pH value observations showed that there was no significant difference in all treatments. However, the results of the DMRT test on the observation of lactic acid contents showed that there was a significant difference between T2 and T3, while the observation of ethanol contents showed a significant difference between control with T1 and T2 with T3.

The treatment effect on the viability of LAB and yeast inside EP

ANOVA test results show that MAG affects LAB and yeast viability ($p<0.05$). The results of the DMRT test on LAB viability observations showed that there was a significant difference between T1 and T2. Whereas, in yeast viability observations showed a significant difference in control with T1 and T2 with T3.

DISCUSSION

This study result show that a decrease in pH leads to an increase in acidity. Titratable acidity is a measurement of milk buffering capacity as an indication of the freshness of milk and fermented milk products. The highest acidity (0.13%) was found in EP made from T3 and the lowest acidity (0.09%) was found in control. These results were different from Goktas *et al.* [10] who used single culture LAB and yeast into ICM resulting in greater acidity of 0.26% and 0.35% respectively. This phenomenon appears because the kefir grains used in this study contain a more complex combination of LAB and yeast. The high acidity value was caused by the abundant lactic acid content.

The increase in acidity of EP occurs because of the increase in the concentration of MAG used. GMK contains probiotics consisting of kefir grains that consisting LAB and yeast. The role of LAB and yeast were to break down fat and lactose into simple compound. Guzel-Seydim *et al.* [5] explain that acidification of milk occurs when probiotic converts lactose into lactic acid. Probiotic activity causes acidification that affects the value of acidity. Increased acidity followed by increased overrun.

Table 1 shows that overrun of EP increases in line with the increasing the concentration of MAG in combination with GMK ($p<0.05$). The highest overrun was found in EP made from T3 and the lowest overrun was found in T0. The results showed that overrun in EP only appeared after the use of MAG by 0.50%. This result was not the same as the results from Singh *et al.* [16] who explain that the increase in overrun value of ice cream starts from 0.23% - 0.32% if using MAG as an emulsifier. This phenomenon occurs because the overrun that appears in the control and T1 does not have a significant difference so that T2 has a more formed overrun.

Overrun is an indicator to determine how much the final volume increase of an ice cream affects its sensory quality. The fat content in milk affects the overrun value of an ice cream. According to Guerrero *et al.* [21], the maximum increase in an ice cream's overrun value was 100%. According to Guimarães *et al.* [22], the more efficient stirring process, the more air enters the ICM. Aiza *et al.* [23] reported that ice cream production using goat's milk kefir can produce overrun values up to 30% due to the low-fat content in it. The fat content in milk is able to trap gas that enters the mixture so that ice cream will expand and have certain sensory qualities.

Overrun observations were made because the ICM material used was low-fat fermented milk. MAG has two legs that function to bind hydrophilic and hydrophobic compounds into 1 unit. The results of this study showed that the combination of MAG and GMK was able to produce an overrun of 32% in T3. An increase in non-fat solids content has the potential to cause an increment in overrun values of EP. MAG helps disperse air into the membrane surrounding fat globules. An even dispersion of globules was characterized by an increase in overrun values. This dispersion causes air to disperse across the surface of the fat globules in the ICM. The use of MAG also increases efficiency in the stirring process.

The results showed that higher concentration of MAG increased the overrun of EP. The overrun value of EP was proportional to their value of acidity. According to Martins *et al.* [6], generally a high overrun value will cause ice cream products to become thicker, have good emulsion compactness, the foam formed more stable, and does not melt easily. Table 1 shows that the calculation of melting rate of EP decreases ($p<0.05$) in line with the increase of MAG's concentration in combination with GMK.

This result study shows that increasing concentration of MAG makes it harder to melt. Increasing the amount of MAG in an ICM allows ease in spreading fat globules. MAG can multiply the air content in the ICM during the stirring process. The more air that enters the ICM will increase the overrun value and viscosity of the ice cream. In addition, more air also has the potential to make ice cream more stable and more melt resistant [24].

The high stability of the emulsion prevents larger ice crystals from appearing more during storage in the freezer. The high frequency of large ice crystals will tend to result in a decrease in the time it takes to melt. This result was in line with Khairunnisa *et al.* [25], who explain that the use of emulsifier aims to increase stability and reduce the chances of creating large ice crystals.

According to Karim and Aider [26], the melting rate was inversely proportional against overrun. The higher the overrun value of an ice cream product reduces their melting rate. The melting rate of EP was affected by their overrun. In addition, the size of the ice crystals formed also affects the stability of EP.

During EP production, the pH value measurement was obtained around 5.7 to 6.2. This result in line with Liu *et al.* [27], that explain if decrement in the pH value indicates an increment in the number of probiotics. This decreasing due to changes in product acidity through fermentation reactions by kefir grains. This phenomenon contradicts the findings Izquierdo-González *et al.* [24], that give recommendation pH value for probiotic-based food products was around 5.5. This conflict caused by differences in probiotic cultures used to produce fermented foods. Arief *et al.* [9] explained that each fermented food has a different pH value depending on the type of strain used. Sarwar *et al.* [18] reported that ice cream made from *S. boulardii* has a pH value of 6.40. Goktas *et al.* [10] reported that ice cream made from a combination of *S. boulardii* and *L. rhamnosus* had a pH value of 5.51.

This study results showed that concentration of MAG in combination with GMK affect ($p<0.05$) the lactic acid contents of EP (Table 2). Even so, the lactic acid content of EP was still below the standard of Codex Alimentarius [3], which said at least 0.6%. This could be because the low level of lactic acid was likely due to the starter incubation time that was less than 12 hours. According to Sarwar *et al.* [18], incubation was carried out for 24 hours to maximize the growth and development of *S. boulardii* CNCM I-745 in combination with inulin. *S. boulardii* was able to form nutrients from existing nutrients, they will continue their growth and development activity. Therefore, the extension of the incubation period allows LAB to spend longer in converting nutrients into energy to proliferate.

Table 2. The effect of the combination of MAG and GMK on the pH value, lactic acid, and ethanol contents of EP

Character	Treatment			
	T0	T1	T2	T3
pH value (pH)	6.20^{a}±0.23	6.17^{a}±0.23	5.82^{a}±0.21	5.79^{a}±0.21
Lactic acid (%)	0.09^{a}±0.02	0.11^{a}±0.02	0.13^{a}±0.02	0.14^{b}±0.02
Ethanol (%)	0.47^{a}±0.03	0.60^{b}±0.03	0.66^{b}±0.02	0.76^{c}±0.02

T0 = GMK + 0.00% MAG + 4% sucrose + 0.1% salt as control after 28 days storage, T1 = GMK + 0.25% MAG + 4% sucrose + 0.1% salt after 28 days storage, T2 = GMK + 0.50% MAG + 4% sucrose + 0.1% salt after 28 days storage, T3 = GMK + 0.75% MAG + 4% sucrose + 0.1% salt after 28 days storage, Numbers followed by different letters on the same line show a significant difference with a significance level of 5%.

Table 3. The effect of the combination of MAG and GMK on the viability of LAB and yeast inside EP

Viability	Treatment			
	T0	T1	T2	T3
LAB (CFU/ml)	1.983×10^{6a} ±78.36	2,101×10^{6a} ±83.03	2,160×10^{6b} ±85.35	2,233×10^{6b} ±88.25
Yeast (CFU/ml)	0.911×10^{4a} ±0.04	1.028×10^{4b} ±0.05	1.070×10^{4b} ±0.05	0.967×10^{4a} ±0.05

T0 = GMK + 0.00% MAG + 4% sucrose + 0.1% salt as control after 28 days storage, T1 = GMK + 0.25% MAG + 4% sucrose + 0.1% salt after 28 days storage, T2 = GMK + 0.50% MAG + 4% sucrose + 0.1% salt after 28 days storage, T3 = GMK + 0.75% MAG + 4% sucrose + 0.1% salt after 28 days storage, Numbers followed by different letters on the same line show a significant difference with a significance level of 5%.

The results of observations on EP after 28 days showed that the concentration of MAG in combination with GMK affected the ethanol content significantly. The order of ethanol content ranging from smallest to largest was T0, T1, T2, and T3 respectively (Table 2). The results of this study were lower when compared to Sulmiyati *et al.* [12] who reported that kefir production using 2% kefir grains resulted in ethanol contents of 0.69 – 1.78%. The low ethanol content possessed by EP was caused by yeast activity stopped during the 28-day storage stage in the freezer. Guzel-Seydim *et al.* [5] explain that lactic acid and small amounts of ethanol produced during goat's milk fermentation contribute to its unique organoleptic properties. According to Wihansah *et al.* [28], ethanol content in fermented products increases along with the increasing of yeast activity.

The results of this study showed that the increase in MAG concentration caused the viability value to be greater even though there was decrease in the observation of yeast viability on T3. This result was in line with the explanation of Adeloye *et al.* [20] which states that yeast viability number were influenced by LAB activity in converting simple sugars in ICM into lactic acid. There was a competition between LAB and yeast for nutrients contained in ICM such as lactose, sugar, and MAG as energy sources.

Another factor besides nutrition was stress that comes from the production process. This study result shows that concentration of MAG affects the viability of LAB and yeast significantly against stress because of production process. According to Hanafi *et al.* [11], influence of coconut residue dietary fiber as an emulsifier helps probiotic dealing with various stresses that arise due to the ice cream production process. According to Wihansah *et al.* [28], stressors could come from the result of friction between ingredients with the ice cream maker machine and the result of cold stress. Even though nutrient needs have been regulated during formulation, it was still not enough to meet the needs of LAB and yeast.

This study result shows that utilization of MAG in different concentration could help LAB and yeast survive during 28 days storage time. Hanafi *et al.* [11] report that the use of emulsifiers was capable to provide probiotic survival number during frozen storage at least 7.70 x 10^{6} CFU/ml. Goktas *et al.* [10] also reported that the use of emulsifiers on *S.*

boulardii in combination with *Lactobacillus* provide probiotic survival number at least 6 log CFU/g and 9 log CFU/g respectively. World Health Organization (WHO) provides standards related to the minimum viability rate of probiotics that can provide health benefits for consumers were around 10^6 to 10^9 CFU/ml for LAB [19] and 10^4 CFU/ml for yeast [3]. The number of LAB and yeast inside EP consecutively still met the standards of the WHO (Table 3).

CONCLUSIONS

In conclusion, the use of MAG 0.50% into formula EP was the best treatment. The use of MAG exceeding 0.50% turned out to produce EP with almost the same acidity, overrun, and melting rate characteristics. In addition, the use of MAG below 0.05% did not show any difference in pH value, lactic acid content, and ethanol content. The use of MAG as much as 0.50% was also able to save yeast viability even though LAB had a smaller amount of viability than T1.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

ACKNOWLEDGMENTS

The authors acknowledge the facilities, scientific and technical support from Animal Product Technology Department, Animal Science Faculty, Brawijaya University. The authors also acknowledge the institute for Research and Community Service through Non-Tax State Revenue of Brawijaya University by the List of Budget Implementation Fills number SP DIPA-023.17.2.677512/2021 for the grant on this study.

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