

CHARACTERIZATION AND PRODUCTION OF GOAT MILK KEFIR- PEPTIDE ON TRIGLYCERIDE SYNTHESIS OF CELL MODEL OF 3T3-L1

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ABSTRACT

Goat milk kefir (GMK) refers to fermented products, generated through fermenting milk with microbial culture called kefir grains. Prior studies have reported the changes in kefir quality properties during aging process in which fermentation continues, resulting in changes of the peptide content. This research aims to investigate the effect of aging time on GMK-peptide characteristics to inhibit triglyceride syntheses on cells model of 3T3-L1. In this study, GMK were stored at 4 ± 1 °C for 0, 2, 4, 6 and 8 weeks, respectively. The protein profile was characterized by implementing SDS-PAGE. The result of experiment indicated no protein degradation during the 6 weeks of aging period particularly for high molecular weight at 84 kDa, 80 kDa and 65 kDa. The GMK-supernatant from 8 weeks storage was performed by applying ultrafiltration membrane cut off < 30 kDa (UFC8 030.08). GMK-filtrate fractions (GMK-peptide) was collected as peptides. After 8 weeks of aging period, kefir protein profile was found to consist of peptide fractions and amino acid. The proteolytic activity of kefir grain increased linearly along with aging time (0-8 weeks). During aging period, the proteolytic activity of grain kefir released peptides and amino acid. In particular, the antioxidant activities were found significantly different ($p < 0.05$) during aging periods. The antioxidant activities of GMK-peptide increased along with the elevating peptide concentration, from 3.30 % to 55.73%, with derivate by GMK-peptides of 2.75 – 10.39 mg/ml. Obesity associated with adipocyte hypertrophy occurred when TG accumulation. GMK-peptide of 100 µg/ml indicated the lowest TG level (2.00 ± 0.03 mg/dl). This finding was in line with the inhibition of TG synthesis (61.14 ± 3.26 %). However, GMK-peptide contained antioxidant potency due to may be correlated with decreasing TG synthesis. The study thus suggested the important role of kefir to prolong aging in generating higher peptide bioactive as antioxidant and inhibition of TG synthesis of cell model of 3T3-L1.

Keywords: Anti-oxidant; fermentation; obesity; proteolytic activity

INTRODUCTION

Goat milk show higher digestibility than cow milk, distinct, higher buffering capacity (alkalinity) and therapeutic effect (Getaneh *et al.*, 2016). Goat milk kefir refers to milk culture, acidified by Lactic Acid Bacteria (LAB) such as *Lactobacillus acidophilus*, *Lb. casei*, *Lb. reuteri*, *Lb. plantarum*, *Lb. bulgaricus*, *L. lactis*, *Bifidobacterium bifidum*, *B. lactis* (Pacheco Da Silva *et al.*, 2016). Free radicals is excess of oxidation reported to be one of the causes of pathogenesis and diseases in humans such as membrane lipids, proteins, enzymes and DNA damage (Rahal *et al.*, 2014). It is consequently to prevent free radicals further reacted with living cells (Chen *et al.*, 2021). Antioxidants as scavenger to free-radical result of oxidation process. Despite exhibiting antioxidant activity of administration of artificial antioxidants against several oxidation systems, is restricted due to the potential risks for the living body. Various antioxidant sources frequent consumption like vegetables, fruit, herbs and spices (Mushtaq, *et al.*, 2020). Similarly, GMK-peptide as peptide short chain, and amino acid exhibited antioxidant properties (Zulleita, *et al.*, 2009; Ceballos *et al.*, 2009). Prior studies (Mahdi, Untari and Padaga, 2018) have identified antioxidative peptides from GMK as well as reported by Li *et al.*, (2013). However, only few reports presented the bioactive peptides derived from GM-kefir exhibiting antioxidative activity during fermentation with grain kefir. GMK-Peptide particularly by applying ultrafiltration membrane cut off < 30 kDa (UFC8 030.08) fraction implemented in the research was isolated

and characterized. Most of studies focused on HPLC fractionation for peptide characterization. This study additionally emphasizes that peptides were found novel due to its antioxidative activity relationship with TG synthesis in cells 3T3-L1 adiposity

Obesity is identified as a chronic disease and defined as the accumulation of fat in the body, leading to various diseases as hypertension, heart disease, osteoarthritis and diabetes (Marseglia, *et al.* 2015). Proliferated cases of obesity emerges in various countries worldwide; thereby alleged as a global pandemic (Francisco *et al.*, 2018), indicating a continuous escalation from year to year. The prevalence of obesity varies greatly among countries influenced by genetic and diet factors (Mohammadi, *et al.*, 2019), inducing cascade reaction of oxidative stress and increasing reactive oxygen species (ROS), by accumulation of triglyceride (TG). Particularly, the prevalence of obesity in Indonesia adult men (> 18 years) was 7.8% (2018), and 19.7% (2013) (RiskeDas, 2018). Whereas in adult women (> 18 years), the prevalence of obesity increased from 17.5% to 32.9 (Panchal, *et al.*, 2020).

Numerous studies have been conducted to prevent and treat obesity, through inhibiting adipocyte differentiation, stimulating exercises, reduce food intake, regulating lipid metabolism, and inhibiting lipase enzyme (Yun, 2010).

GM-Kefir and its active chemical constituents are reported to contain beneficial property and activity for the reducing of obesity. Therefore, applying kefir is take consideration effective as natural medicine with high safety compared to synthetic drug. Kefir from fermented goat milk contains high bioactive compound such as peptides (Fan, *et al.*, 2018). Other

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research reports have indicated that peptides from GMK play in reducing body fat and lipid metabolism control (Zeybek *et al.*, 2019). Purified tripeptide exhibited inhibition adipogenesis of 3T3-L1 with IC_{50} value of 14 $\mu\text{g/ml}$. Similarly, flavoenzyme hydrolysate from soy protein isolate possessed a lipolysis-stimulating activity by measuring glycerol release in 3T3-L1 adipocytes. Further, GMK-peptide was reported to contain anti-hypertension and antioxidant. However, effort to update anti-obesity peptide derived from GMK-peptide is not identified yet. Thus, this study aims to identify GMK-peptide as antioxidants and inhibitory effect of TG accumulation in cell model of 3T3-L1.

MATERIAL AND METHODS

Whey Kefir Protein Profile Based on the Gel Electrophoresis

GM-Kefir was supplied by Bio-health small industry kefir production in Malang, Indonesia. The samples were stored at 4-6 °C for 0-8 weeks as aging period. After 8 weeks, GMK-kefir was centrifuged at 4500 rpm for 15 minutes to collect the supernatant of whey protein, performed by utilizing SDS-polyacrylamide gel electrophoresis to characterize the GMK-whey profile before and after aging period for 0-8 weeks. Gel polyacrylamide with eluent buffer consisted of 0.112 M acetic acid, 0.112 M Tris, and pH of 6.5, and buffer strip in 3% agarose with 0.20 M tricin, 0.20 M Tris, 0.55% SDS with pH of 8.1. Molecule standard content indicated that sample solution containing 15 μl of each sample buffer and run in 4% Bis-Tris for 2 hours at 120 V of voltage and 4°C of temperature. The relative band protein fraction migration was measured to determine the molecule weight (MW), by comparing with MW bandmarker line (Walker, 1996).

Peptide Determination

Upon the completion of a 8 week storage, GMK-peptide was fraction from GM-kefir by centrifuged at 4500 rpm for

15 minutes, then the supernatant was performed using ultrafiltration membrane cut off < 30 kDa (UFC8 030.08). Further, kefir filtrate fractions (GMK-peptide) was collected, dried freeze and stored at -20°C for later activity assay.

The peptide concentration was measured by OPA (O-phthalaldehyde) method using tryptone standard (Febrisiantosa, *et al.*, 2013). In addition, reagents including 25 ml 100 mM Sodium tetra borax, 2,5 ml 20% (g/g) sodium dodecyl sulfate (SDS), 1,1 ml (40 mg OPA in 1 ml methanol with 100 ml β -mercaptoetanol), and 21,4 ml H₂O were utilized. Approximately 30 μl of GMK-peptide fraction (10 mg/ml protein) was added with 1 ml reagent OPA, and incubated for 2 minutes, to measure the absorbance at λ_{340} nm. Peptide levels were estimated with various Triton standard curve concentrations (0; 0.25; 0.5; 0.75; 1.00; 1.25; 1.50; 1.75; 2.00; 2.25; 2.50 mg/ml).

Determination of Amino Acid

In this study, amino acid were measured by HPLC (Knauer) according to Marino, *et al* (2010) with some modifications, including HPLC condition: Eurospher 100-5 C18 column of 250 x 4,6 mm with prep-column of P/N; 1115Y535; Eluent A: 0.01 M Acetic buffer with pH of 5.9 and B: MeOH: 0.01 M Acetic buffer pH 5.9 and tetrahydro folic (THF) by ratio (80:15:5); and Detector fluorescence (Excitation. λ 340 nm; Emission 450 nm). The sample elution was performed at a flow rate of 1.5 mL/min. In detail, 5 g of sample was prepared by weighing and inserting into a tube to be covered. Hydrochloric acid 6 N was added as much as 10 ml and vortex until exhibiting homogenous condition.

Hydrolysis of sample was performed by using autoclave at 110 °C for 8 h. Sample was cooled at room temperature and neutralized with NaOH 6 N. Solution of 40% Pb (CH₃COO)₄ and 15% oxalic acid were added and inserted into the vial and diluted with aquadest until 50 ml. Further, approximately 3 ml of sample was obtained

and filtered with 0.45 µm cover. Sample was later diluted for 10 times and incubated for 3 min in OPA. Sample was injected with as much as 30 µl to HPLC (Nurliyani et al., 2014)

Antioxidant activity of DPPH⁺ free radical scavenging activity assay

Antioxidant activity of DPPH⁺ free-radical was determined by Mc Cue and Shetty (2005) was considered to evaluate the

antioxidant activity of the bioactive compounds, by measuring the decrease in absorbance of DPPH⁺ at 517 nm (Daichi spectrophotometer UV-2000-1), which was in accordance with Mc Cue and Shetty (2005) with some modifications, included the addition of 4.5 ml methanol to 0.5 ml of DPPH⁺ solution (about 0.5 g/L) and 0.1 mL of purified sample. Standard index such as BHT was used as a reference index to determine radical scavenging capacity of the obtained sample, calculated as:

$$\text{Radical scavenging activity (\%)} = (A_{t_0} - A_{t_{\text{end}}}) / A_{t_0} \times 100\%$$

Where :

A_{t_0} refers to the absorbance at time zero

$A_{t_{\text{end}}}$ refers to the absorbance after 60 min.

Cell culture and adipocyte differentiation

Cell model of 3T3-L1 was supplied from Cell Culture and Cytogenetic Laboratory, Faculty of Medicine Pajajaran University, Bandung Indonesia. The cells were cultured in preadipocyte medium (DMEM) (Gibco-11995065) with high glucose and pyruvate, supplemented with 10% of Fetal Bovine Serum (FBS) (Gibco-10270106), 1% Antibiotic/Antimycotic (ABAM) (Gibco-15240-062), further incubated for 24 hours at 37 °C and 5% CO₂. Culture medium was shifted every two day period. After the preadipocyte cells reaching to 70-90%, confluences were stimulated with 1 µl differentiation Cocktail K579-100-1 (1.5 µg Insulin; 1 µM Dexamethasone; 1 µM 3-Isobutyl-1 methylxanthine), later incubated for 24 hours at 37 °C and 10 % CO₂. The process was continued by shifting the medium and continuing the incubation until 7 days (Torous, et al., 2020).

Triglyceride (TG) Measurement

The 3T3-L1 adipocytes were harvested in 7 days after initiation of differentiation, treated with (GMK-peptide) consisting of positive (3T3-L1 with differentiation Cocktail) and negative control (3T3-L1 without differentiation Cocktail), the experiment

consist of 3T3-L1 with differentiation Cocktail and each sample was adding with GMK-peptide 25, 50, 75 and 100 µg/ml sequentially. The cells were washed once with cold PBS. Cells were collected, and lysed in 100 µl of lysis buffer (K610-100-2) cover to prevent evaporation. The cells were plate-heated at 90-100°C for 30 min generating cloudy solution during heating process. Later, the process was progressed by plate cooling the solution to room temperature. The solution was further mixed by shaking plate for 1 minute. TG completely dissolved in the lipid extraction buffer, transferring 50 µl of lipid extracts to 96 wells plate added with 50 µl of the reaction mix to each well containing TG standard, sample and control. Upon mixed, the solution was incubated at 37 °C for 30 minutes to measure the OD 570 nm (Kim, et al., 2006). TG concentration was calculated with: $C = T_s / S_v$ (nmol/ul), where T_s denotes TG amount from standard curve (nmol), S_v denotes the sample volume (before delusion) added in sample wells (µl).

Statistical Analysis

In this study, data are presented as mean value with standard deviation. The significance between mean values was determined statistically with variance

analysis in 5 different aging periods with 4 replications. Results of statistical analysis are reported as means \pm standard deviation.

RESULT AND DISCUSSION

The Effect of Aging Period on Kefir Whey Protein Profile

According to the manufacturer of goat milk kefir manufacture, the final acidification degree of kefir should reach pH of 4.6. In this study, after 8 week aging period, kefir was centrifuged at 4500 rpm for 15 minutes. The supernatant is kefir whey containing soluble protein or peptide. Whey kefir protein indicated decreasing pH after 8 week aging period affecting the protein fermentation and releasing peptides. The pH degree of whey kefir protein could limit or modify the accessibility to some cleavage site for grain kefir enzymes (Kopf-Bolanz *et*

al., 2014). The SDS-PAGE profiles of kefir whey are illustrated in Fig.1. This study however did not observe protein degradation during a 6 week aging period due to indicating similar protein profiles particularly for protein with MW at 84 kDa, 80 kDa and 65 kDa. Thus, it is suggested that this short fermentation did not exhibit protein degradation which could be seen by SDS-PAGE. However, after 8 week aging period, the protein profile indicated a decreasing high molecule weight band protein intensity. Through the fractionation process of whey protein by ultrafiltration, milk casein were degraded into smaller fragments, despite leaving a remaining intact α -casein, β -casein and β -lactoglobulin (Egger, *et al.*, 2016). After 8 week aging period, the result indicated that kefir protein profile exhibited lower MW as peptide fractions.



Figure 1. Whey kefir protein profile after aging period at 4°C for (0, 2, 4, 6 and 8 weeks)

Table 1. Amino acid composition (mg/ml) of peptides after aging period at 4°C.

Composition	Aging (weeks)				
	0	2	4	6	8
Aspartic acid	0.157 ^a ±0.002	0.360 ^b ±0.012	0.903 ^d ±0.013	0.503 ^c ±0.015	0.500 ^c ±0.000
Glutamic acid	0.025 ^a ±0.007	0.430 ^b ±0.001	0.435 ^b ±0.010	0.500 ^b ±0.000	0.570 ^b ±0.000
Serine	0.036 ^a ±0.010	0.200 ^a ±0.000	0.690 ^c ±0.001	0.180 ^a ±0.000	0.189 ^a ±0.024
Histidine	0.301 ^b ±0.000	0.120 ^a ±0.000	0.236 ^a ±0.004	0.360 ^b ±0.010	0.360 ^b ±0.027
Glycine	0.030 ^b ±0.000	0.340 ^b ±0.003	0.126 ^a ±0.006	0.400 ^c ±0.020	0.421 ^c ±0.023
Threonine	0.053 ^a ±0.013	0.140 ^a ±0.000	0.602 ^b ±0.014	2.000 ^c ±0.040	2.050 ^c ±0.025
Arginine	0.045 ^a ±0.002	0.010 ^a ±0.000	0.194 ^a ±0.001	1.000 ^b ±0.050	2.000 ^c ±0.202
Alanine	0.072 ^b ±0.004	0.096 ^c ±0.001	0.040 ^a ±0.000	0.264 ^d ±0.021	0.262 ^d ±0.023
Tyrosine	0.133 ^a ±0.012	0.420 ^b ±0.015	0.930 ^c ±0.017	0.430 ^b ±0.016	0.432 ^b ±0.031
Methionine	0.621 ^c ±0.003	0.329 ^b ±0.004	0.560 ^c ±0.012	1.012 ^d ±0.102	0.027 ^a ±0.061
Valin	0.106 ^a ±0.020	0.240 ^b ±0.002	0.410 ^c ±0.014	0.650 ^d ±0.030	0.653 ^d ±0.024
Phenylalanine	0.070 ^a ±0.001	0.300 ^c ±0.002	0.144 ^a ±0.003	0.490 ^d ±0.023	0.230 ^b ±0.160
Isoleucine	0.062 ^a ±0.003	0.410 ^c ±0.001	0.210 ^b ±0.002	0.200 ^b ±0.001	0.220 ^b ±0.017
Leucine	0.593 ^b ±0.007	0.231 ^a ±0.004	0.200 ^a ±0.002	0.710 ^c ±0.050	0.310 ^a ±0.024
Lysine	0.494 ^b ±0.001	0.210 ^a ±0.002	0.040 ^b ±0.001	2.000 ^c ±0.100	2.000 ^c ±0.013

*Data are presented as Mean ± Standard Deviation. Different superscript letter shows significant difference (p<0.05) among treatments.

The decreasing concentration of high molecule weight component along with the increasing peptides in whey kefir protein indicated that kefir grain and their metabolites enzymes had fermented the protein further in aging at 4°C. Kefir whey protein from different aging period contains various amounts of amino acid (illustrated in Table 1) such as, serine, isoleucine, phenylalanine, lysine and glutamic acid increasing during aging period, as the result of composition variability generating various bioactive properties of GMK-peptides.

Effect of Aging on Proteolytic Activity

Milk protein are recognized as one of sources for bioactive peptides. Table 1 show that several of amino acid increased

significantly during aging periods, such as aspartic acid, glutamic acid, threonine, and arginine, but the other trend decreased. According to Panchal., et al (2020) and Wang et al., (2021) have been reported in goat milk and fermented goat milk products abundant of bioactive peptides. In addition-kefir is known for its health promoting effects and there are more than 1500 peptides identified in milk kefir, reflecting the proteolytic activity of the microbial culture (Dallas et al., 2016). Proteolytic activity differs significantly (p<0.05) among aging period. The mean proteolytic activity of kefir culture increased linearly (r = 0,99) along with aging period (0-8 weeks) (Table 2) depicted during aging period in releasing peptides and amino acid associated with proteolytic activity.

Table 2. Effect of aging period on peptide (mg/ml), proteolytic activity (mg/ml) and DPPH⁺ free radical scavenging activity (%)

Aging Period (Time in weeks)	Proteolytic Activity (mg/ml)	Peptide < 30 kDa (mg/ml)	Radical scavenging activity (%)
0	1.68 ^a ±0.05	2.78 ^f ± 0.11	3.30 ^k ±0.11
2	2.76 ^b ±0.20	3.82 ^g ±0.02	5.08 ^l ±0.47
4	3.28 ^c ±0.01	5.22 ^h ±0.34	28.70 ^m ±1.15
6	5.37 ^d ±0.01	8.44 ⁱ ±0.30	40.60 ⁿ ±2,70
8	7.44 ^e ±0.03	10.39 ^j ±0.21	55.73 ^o ±1.85

*Data are presented as Mean±Standard Deviation. Different superscript letter (a-c) (f-j) (k-o) show significant difference (p<0.05) among treatments.

Antioxidant Activity of DPPH⁺ Free Radical Scavenging Assay of GMK-peptide

Antioxidant activities of DPPH⁺ free radical scavenging assay of GMK-peptide was presented in Table 2, indicating that antioxidant activities differed at ($P < 0.05$) in aging period from 0 to 8 weeks. The antioxidant activities of GMK-peptide increased simultaneously along with the increasing peptide concentration at 8 week aging period (from 3.30 % to 55.73% derivate by GMK-peptides of 10.39 mg/ml), which was also presented by Rahmawati and Suntornsu (2016), highlighting that antioxidative activity of 19% in goat milk yogurt increased during storage at 4°C up to 21 days.

However, this finding was in contrast with another study (Radiati, *et al.*, 2012; 2016) indicating the decrease after 21 days of storage. Similarly, Panchal *et al* (2020) also measured ABTS activity of GM-beverage by microbial culture and addition with grape pomace on gut microbiota and antioxidant activity. GM-yogurt with grape was 418.02 ± 16.14 mmol TE/g and goat milk with grape juice was 743.78. Chen *et al* (2019) investigated that cheddar cheeseshow the increasing antioxidants activity during ripening in the first 4 weeks of storage ($P < 0.050$, and

reached the maximum of 43.86% to 47.43% which was higher than control cheese. Such finding was in accordance with another study following the antioxidant activity of amino acids, released during the fermentation process such as cysteine, tryptophan and methionine (Elias *et al.*, 2005).

This study, particularly, reported a significant increase of DPPH⁺ free radical scavenging activity than those in control (0 week). Another study (Liu, *et al.*, 2016) indicated GMK-peptide could extend the proteolysis with maximum antioxidant activity of peptides in this experiment to be 55.73 ± 1.85 % (Table 2).

Effect of GMK-peptide on Triglyceride Level on Cell Model of 3T3-L1

Cell model of 3T3-L1 refers to cell model of obesity. Obesity can induce oxidative stress leading to free radicals, thereby requiring antioxidants to reduce free radical levels. Fermented milk provides a source of antioxidants in the forms of protein derivatives with low molecular weight having antioxidant activity. In this study, fermented peptides were isolated by centrifugation and filtration to obtain GMK-peptides with molecular weights below 30 kDa, illustrated in Table 2.

Table 3. Level of triglyceride in adipocyte (3T3-L1) treated with GMK- peptide

Samples	TG(mg/dl)	Inhibition of TG level over positive control (%)
Positive Control	$51.48^e \pm 2.05$	$0.00f \pm 0.00$
Negative Control	$17.5^a \pm 1.01$	$66.00y \pm 2.06$
GMK- Peptide 25 µg/ml	$38.12^d \pm 1.57$	$25.99g \pm 2.11$
GMK- Peptide 50 µg/ml	$25.72^c \pm 1.16$	$50.03h \pm 3.20$
GMK- Peptide 75 µg/ml	$20.10^b \pm 1.17$	$60.95i \pm 3.13$
GMK- Peptide 100 µg/ml	$20.20^a \pm 0.13$	$60.76i \pm 3.26$

*Data are presented as Mean \pm Standard Deviation. Different superscript letter (a-c) show significant difference ($p < 0.05$) among treatments (Control Negative: un-differentiated, Control Positive: differentiated cells, GMK-peptide treatment) in TG level and inhibition TG level over positive control show significant difference, the data was analyzed with Anova and Duncan between treatment

The finding presents that higher peptide concentration resulted in higher free radical scavenging in line with the inhibition of TG synthesis. GMK-peptide has anti-inflammatory agent (Raras, *et al.*, 2015), antioxidant agent, antiadipogenesis which are advantageous in preventing obesity. GMK-peptide (Table 3), inhibited of the transcriptional regulation of lipid synthesis or stimulation of lipolysis in 3T3-L1 adipocytes. The pre-adipocyte differentiated into adipocytes is regulated by a complex network of transcription factors. After differentiation, C/EBP β was induced immediately, while C/EBP α and PPAR γ serve as master regulators of adipogenesis which is critical to the progression of the final stages of adipocyte differentiation. In general, differentiation of adipocyte and fat accumulation are associated with the obesity development.

The increasing number of fat cells and adipose tissue mass could further cause obesity. Theoretically, the TG formation was measured by optical density changing, thereby generating of lipid droplets formation was measured by colorimetric assay, the higher TG resulting in higher absorbance, effective in the induction of differentiation of pre-adipocyte to adipocyte.

with negative control indicated "undifferentiated" as marked by the lowest TG level in colorimetric reading. There was difference between undifferentiated cells (Negative Control with the differentiated cells (Positive Control). In this research, GMK-peptide was evident to reduce TG level of 3T3-L1 (66.00 ± 2.06 %). Furthermore, higher TG level in adipose tissues increased the oxidation in cells level. Higher TG in plasma serum is correlated with obesity. In the current study, GMK-peptide was imminent to reduce TG level in cells of 3T3-L1 adiposity. Increasing the TG induced the fatty Acid Synthase (FAS), Acetyl-CoA Carboxylase (ACC) and adipocyte-specific fatty acid binding protein (aP2) genes expression. The decreasing TG content was resulted from the decreasing re-

esterification of FFA. Peptide was also found to decrease PEPCK level, playing a role in adipogenesis through induced the ligand Peroxisome Proliferator-Activated Receptor γ (PPAR γ) which contributed to induce of adipocyte-specific gene expression and differentiation by controlling the fat accumulation in the adipose tissue mass associated with obesity development. These results suggest that GMK-peptide contains anti-adipogenesis effect, lowering body weight as well as lowering FAS concentration in adipose tissue and serum of experimental rats. Thus, anti-obesity potency of GMK-peptide is strongly potential to its amino acid content based on HPLC assay.

This finding is in accordance with another study (Kim *et al.*, 2017) emphasizing that peptide exhibited anti-obesity activity through antioxidant activity, also supported by other studies reporting the role of peptide in reducing lipid accumulation.

CONCLUSION

In sum, this study presented the role of kefir in addressing the obesity by investigating several components of kefir storage. It was also reported that aging period contributed to proteolytic activity following the increasing peptide and antioxidant activities to offer synergistic efficacy in blocking cellular oxidation mechanism. Subsequently, GMK-peptide was found to be potential in preventing fat accumulation through inhibiting molecular pathway of lipid.

Disclosure statement

The authors declare no conflict interest

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