Nutrition Quality and Microbiology of Goat Milk Kefir Fortified with Vitamin B$_{12}$ and Vitamin D$_3$ during Storage

EP Dianti $^{1}$, G Anjani$^{1,2}$, DNAfiah$^{1,2}$, N Rustanti$^{1,2}$, B Panunggal$^{1,2*}$

$^{1}$Department of Nutrition Science, Faculty of Medicine, Diponegoro University, Jl. Prof. H. Soedarto,SH, Tembalang, Semarang 50275, Indonesia

$^{2}$ Center of Nutrition Research (CENURE), Diponegoro University, Diponegoro University, Jl. Prof. H. Soedarto,SH, Tembalang, Semarang 50275, Indonesia

Corresponding author, +62 81285376785, panunggalbinar@gmail.com

Abstract Goat milk kefir fortified vitamin B$_{12}$ and vitamin D$_3$ can be an alternative to improve insulin resistance. Fortification of vitamin B$_{12}$ and vitamin D$_3$ could affect the balance of complex ecosystems of bacteria and yeasts in goat milk kefir. We analyzed the nutritional quality and microbiology of goat milk kefir during storage. This experiment was done with completely randomized design consisted of five treatments, ie storage day 0, 3$^{rd}$, 6$^{th}$, 9$^{th}$, and 15$^{th}$. Vitamin B$_{12}$, vitamin D$_3$, protein were analyzed using spectrophotometric, fat using babcock method, total lactic acid bacteria using total plate count, pH with pH meter, and viscosity using ostwald method. During 15 days in refrigerated storage, there was no significant difference (p>0.05) of vitamin B$_{12}$ concentration, protein, fat, and viscosity of kefir. Storage time of the final product caused the decrease of vitamin D$_3$ concentration between day 9 and 15 (p=0.038), pH (p=0.023), and total lactic acid of kefir (p=0.0001). Total lactic acid bacteria increased until 9 days of storage (6161 ± 1.296x10$^7$cfu / ml), but decreased to 24% on day 15 (150 ±7.78x10$^7$cfu / ml). pH kefir tends to fluctuate during storage with the highest pH value of 4.38 on day-3, and lowest 4.13 on day-9.

Keywords: goat milk, kefir, storage, fortification, vitamin B$_{12}$ and vitamin D$_3$

1. INTRODUCTION

Indonesia is a developing country that has a tropical climate, is now facing new health problem. The epidemiological transition has resulted in a double burden of infectious diseases and chronic non communicable diseases [1]. Nowadays, diabetes has become a major public health problem in Indonesia and needs national strategies to screen, prevent and treat the disease [2]. Insulin resistance is the stage of diabetes mellitus. This is a condition of cells or tissues which could not use insulin effectively to regulate the transport of glucose into target cells or tissues resulting in impaired blood glucose absorption[1]. Riskesdas 2013 reported 36.6% of Indonesian population aged >15 years old experienced fasting blood glucose state disrupted, this condition lead to diabetes mellitus type 2.

Based on study, patient with insulin resistance tend to have low level of vitamin D3 and vitamin B12 in the plasma [3-5]. Vitamin D supports pancreatic beta cell activity. Beta cells possess vitamin D receptors. Animal studies show that missing vitamin D receptors result in poor insulin secretion and vitamin D supplementation improves insulin secretion[3]. Vitamin B12 deficiency is well documented in adults with inadequate intake, gut malabsorption or pernicious anaemia. Malabsorption of B12 is also
associated with metformin therapy, an insulin sensitizer used for the treatment of type 2 diabetes and increasingly in obese, insulin resistant, adolescents.[4-5]

Food fortification is an effort to prevent vitamin B12 and D3 deficiency and improve insulin resistance through serving food which rich of vitamin B12 and vitamin D3. The success fortification depends on the choice of fortified media. Fortification vehicle should be easy to obtain, affordable prices, and contain high nutritional value[6].

Goat milk kefir is a good vehicle for fortifying vitamin B12 and vitamin D3 because it has high nutritional value [7]. It has been proven to have many health benefits, such as maintaining the balance of intestinal microflora, strengthening the immune system, lowering blood sugar and cholesterol[8]. Kefir is a type of dairy fermented from kefir grain containing probiotic bacteria complexes, such as lactobacilli, lactococi, leuconostoc, and acetobacteria and yeast[7]. The taste of the natural kefir beverage has been described as a yeasty taste, sparkling and pricking have been used to describe the mouth-feel of the kefir beverage caused by the liberation of CO2. The distinctive taste of kefir is a result of several flavour compounds which are produced during fermentation. In this study, kefir is made of goat milk. Goat milk has the advantage of rich minerals of calcium, phosphorus, magnesium, vitamin A, vitamin C and medium chain tryglyserides (MCT) rather than cow's milk. However, goat milk contained only slight vitamin B12 and D3[9].

Fortification of vitamin B12 and D3 affect the quality of nutrition and microbiology because goat milk kefir contained complex of probiotic and yeast bacteria that are active and sensitive to environment changes. Previous study reported that there was an increase in CO2 and ethanol due to Saccaromyces cerevisiae activity during storage[10]. In addition, the activity of lactic acid bacteria and [11-12]. In this study, the nutritional quality of goat milk kefir vitamin B12 and vitamin D3, which includes vitamin B12, vitamin D3, carbohydrates, proteins, fats, and total bacteria during storage of day 0, 3, 6, 9, and 15 were analyzed.

2. MATERIALS AND METHODS

2.1 Preparation of Goat Milk Kefir Fortified with Vitamin B12 and Vitamin D3

The main ingredients of goat milk kefir were kefir grain, goat milk, vitamin D3 and vitamin B12. Goat's milk and kefir grain were obtained from Omah Kefir Ungaran. Vitamin B12 was obtained from the nutritional support product “Blackmores Vitamin B12”, while vitamin D3 is obtained from the product of “Vitamin D3 1000 IU Healthy Care Australia”.

The first step to make kefir kefir was pasteurized of goat milk up to 70°C. Furthermore, goat milk cooled until 25°C, then inoculated with kefir grain (5% of the total milk). Fermentation duration was 24 hours and it stirred every 6 hours once. Fortification of vitamin B12 20 mcg / 100 mL and vitamin D3 42 IU / 100 mL at 12 hours fermentation[13-14]. After fermented 24 hours, then kefir filtered to separate kefir and kefir grain. Furthermore, kefir was divided into 5 groups of storage treatment ie kefir day 0, 3, 6, 9, and 15 for tested vitamin B12, vitamin D3, protein, fat, total lactic acid bacteria, pH, and viscosity.

2.2 Nutrition quality assay

Vitamin was analyzed by spectrophotometry. The wavelength of vitamin B12 was 530 nm and 264 nm for vitamin D3. Determination of protein concentration using Bradford method then analyzed by spectrophotometry at wavelength 595 nm. Preparation of bradford reagent was done by dissolving 10 mg of coomassie brilliant blue G-250 in 5 mL of 95% ethanol. Then, 10 mL of 85% phosphoric acid was added. The solution was diluted with distilled water to a volume of 100 mL. The samples (each 0.1 g) were added bradford reagent, then it was incubated for an hour. The solution was homogenized using a vortex an it was incubated at room temperature for an hour. Fat was analyzed using the babcock method, total lactic acid bacteria with total plate count method using MRSA, viscosity, and pH using pH meter.
2.3 Analysis Data
All data were processed using statistical software. The data were tested for normality using the Sapiro-Wilk test, then the data were tested with anova oneway test, Kruskall Wallis test, and Mann Whitney test based on normality test results.

3. RESULTS
The nutrition quality of goat milk kefir fortified with vitamin B12 and D3 during storage is presented in Table 1.

Table 1. Nutrition quality of goat milk kefir fortified with vitamin B12 and D3 during storage

| Time storage | Mean Vitamin D3 (IU)** p=0.038* | Median Vitamin B12** (µg) p=0.261 | Mean Protein (%)* p=0.019 | Mean Fat (%)* p=0.259 |
|--------------|----------------------------------|------------------------------------|---------------------------|-----------------------|
| Day-0        | 57.63 ± 9.590*a                  | 678.12                             | 2.320 ± 0.268             | 7.0443 ± 0.43935      |
| Day-3        | 44.83 ± 1.1946*ab                | 609.06                             | 1.880 ± 0.1149            | 8.0028 ± 2.3191       |
| Day-6        | 41.16 ± 4.3787*bc                | 560.62                             | 1.595 ± 0.3517            | 8.0151 ± 0.6965       |
| Day-9        | 45.58 ± 6.0774*bc                | 836.25                             | 1.615 ± 0.5567            | 7.3100 ± 1.1399       |
| Day-15       | 27.33 ± 6.3024*c                 | 867.81                             | 1.449 ± 0.3911            | 7.9505 ± 0.8298       |

*Tested with oneway anova
** Tested with Kruskall wallis

Data in table 1 showed that Vitamin B12, protein, and fat remained fairly stable over the period of the study. Storage time of the final product caused the decrease of vitamin D3 concentration. There was significant difference at day-0 to 3rd day (p=0.05) and 9th to 15th day (p=0.05).

Table 2. Viscosity, pH, and total lactic acid bacteria of goat milk kefir fortified with vitamin B12 and D3 during storage

| Time storage | Mean Viscosity (cm²/s)* p=0.038 | Mean pH* p=0.023 | Total lactic acid bacteria *(cfu/mL) p=0.000 |
|--------------|---------------------------------|------------------|---------------------------------------------|
| Day-0        | 0.0429                          | 4.28 ± 0.0461 a  | 5.41 ± 4.419*ad                            |
| Day-3        | 0.0500                          | 4.38 ± 0.05774 b | 53.38 ± 6.301*ad                            |
| Day-6        | 0.0555                          | 4.30 ± 0.0288 ac | 6982 ± 8.732*b                             |
| Day-9        | 0.0470                          | 4.13 ± 0.0346 d  | 6161 ± 1.296*bc                            |
| Day-15       | 0.0446                          | 4.26 ± 0.0416 ac | 150 ± 7.78*d                                |

*Tested with oneway anova
** Tested with Kruskall wallis

Results on viscosity, pH, and total lactic acid bacteria of kefir during storage are presented in table 2. There was significant difference on pH and total lactic acid bacteria. The highest of total lactic acid bacteria was on 6th day, and the lowest on day-0. It had been reported that total lactic acid bacteria got reduced between 9thday and 15th day. pH kefir tend to fluctuate during storage, there was significant different on day-0 to 3rd day, 3rd day to 6th day, and 9th day to 15th day. Meanwhile, viscosity remained stable until the end of storage time observation.

4. DISCUSSION
4.1 Vitamin B12
Kefir fortified vitamin B12 resulted in 40 times higher in vitamin B12. The high concentrate of Vitamin B12 produced by biosynthesis of bacterial fermentation [15-16]. Propionibacterium shermani B369 and Propionibacterium freudenreichi spp were bacteria that produce vitamin B12 during fermentation. The role of vitamin B12 is as an essential cofactor in fatty acids, amino acids, carbohydrates, and nucleic acids[17].

There was no difference of vitamin B12 in goat milk kefir fortified with vitamin B12 and vitamin D3 during storage. The stability of vitamin B12 was affected by enhancement propionic acid bacteria.
which produced vitamin B12. Increasement of propionic acid bacteria followed also by increasement of ethanol, acetic acid, butiric acid, glucose, dan galactose[18].

4.2 Vitamin D3
The result of vitamin B12 was different from vitamin D3. There was difference concentration of vitamin D3 during storage. Vitamin D3 slightly decreased between day 9 to 15. The highest concentration vitamin D3 on day-0 (57,63 ± 5,590 IU) and the lowest on day-15 (27,33±6,3024 IU). Fortified goat milk kefir had higher concentration of vitamin D3 than non-fortified kefir. This results was different from the result of fortification vitamin D3 on yogurt, whereas vitamin D3 was stable until 4 weeks storage[19]. Vitamin D3 was stable in yogurt due to the bacterial in yogurt was not as complex as that in kefir consisted of bacteria and yeast that had ability to proteolisis and lipolisis. Degradation of protein might affected by the stability of concentration of vitamin D3 because vitamin D3 was binding to β-laktoglobulin dan β-kasein (peptide). β-laktoglobulin A and β-kasein were rich in whey and casein.[20]. β-laktoglobulin A and β-kasein was binding vitamin D3 strongly. Interaction of vitamin D3 and protein was dependable each other. Vitamin D3 could be binding to β-lactoglobulin and β-kasein even in the gastric environment[21]. However, the proteolisis activity of complex bacterial and yeast cause degradation β-laktoglobulin A and β-kasein being arginin and glutamin. It affected on instability of vitamin D3[11][33].

In this study, total of lactic acid bacteria decreased during storage, indicated there was increasement of yeast oxidative activity which produced ethanol, Vitamin D3 was sensitive to oxidation. Other factors that affected vitamin D3 instability were light and lipolisis. Vitamin D3 was sensitive on light and vitamin D3 dissolve in lipid, whereas lipolisis might be impaired the stability of vitamin D3[23].

4.3 Protein
Dissolved protein or often called protein digestibility was the ability of a protein to be hydrolyzed into amino acids by digestive enzymes. Protein digestibility is one of the factors that determine protein quality because it shows the availability of amino acids biologically[24]. In this study, the dissolved protein concentration of goat milk kefir vitamin B12 and vitamin D3 fortified stable for 15 days storage. The results of this study were in accordance with studies on kefir without fortification which mentions no difference in protein concentration during storage[12].

During fermentation, protein hydrolysis had occurred by the activity of proteolytic enzymes that break down proteins into smaller protein fractions or peptides. Lactoglobulin and lactalbumin and other components contained in whey will be converted into several peptides. Lactoglobulins which are the main components of whey are converted into β-lactoglobulin-A, β-lactoglobulin-B and angiotensin converting enzyme (ACE). Breakdown of protein into smaller fractions during fermentation caused the value of protein kefir stable during storage because it is degraded during fermentation[24-25].

4.4 Fat
Fat of goat milk kefir did not change significantly during storage. The fat concentration remained stable, although there was an increase in lactic acid bacteria up to the 9th day. Previous study reported the growth of lactic acid bacteria will cause lipolysis of fat to be free fatty acids[26]. The results of this study differ from other studies which suggest that long storage may lead to fat loss and viscosity[11]. Previous study reported fat concentration decreased by 7.9% on day 14, occurs due to the growth of bacteria and yeasts that are lipolisis[26,27]. The type and quality of milk were factors that affects the stability of fat so that the results obtained different from previous study[26].

4.5 Lactic acid bacteria
Kefir and kefir grain contain a wide variety of lactic acid bacteria that had been identified through physiological and chemical tests. There were Lactobacillus acidophilus, Lb. Brevis, Lb. Paracasei subsp. Paracasei, Lb. Delbrueckii subsp, Lb. Helveticus, Lb. Kefiri, Lb. Kefiranofaciens, Lb. Plantarum, Leuconostoc mesenteroides subsp., Lactococcus lactis subsp, Streptococcus thermophilus [28]. The
highest total lactic acid bacteria was obtained on day 6 (6.96 x 10^10 cfu/mL) and the lowest on day 0 (5.31 x 10^7 cfu/mL). Increased lactic acid bacteria made drastically affect the physical appearance of kefir, namely the separation of the mixture (clear yellow and milk white) which showed that there was degradation of macro nutrients by lactic acid bacteria[8]. At day 0, the amount of lactic acid bacteria just a little because lactic acid bacteria was still in the adaptation phase[11]. Lactic acid bacteria populations began to increase on day 3, lactic acid bacteria were able to adapt and utilized the energy source of lactose, yeast would also take the opportunity to hydrolyze lactose, resulting in CO2 and alcohol. The OH-compound of the alcohol reacted with the H* from lactic acid[8][12]. Increased population of lactic acid bacteria continued to day 6 and 9, but there was a decrease in lactic acid bacteria on day 15 due to the activity of yeast metabolism that degraded lactose[10]. This result is in line with other studies which suggest that lactic acid bacteria decreased on day 7 to day 14 because of the increased in yeast[27].

4.6 Acidity (pH)
pH of goat milk fortified with vitamin B12 and vitamin D3 differently during storage. However, the pH of goat's milk fortified with vitamin B12 and vitamin D3 was still considered good and met the pH standard of kefir which ranged from 4.2 to 4.6[16]. The stability of the pH value was influenced by lactic acid bacteria. The more population of lactic acid bacteria, the pH value would be decreased. Differences in pH values occurred from the first 3 days. There was an increased in pH of kefir from 4.28 on day 0 to 4.38 on day 3. This occurred due to the activity of yeasts which changed lactose into alcohol. Based on the previous study, yeast population in kefir increased in the first 7 days[21]. Furthermore, the pH of kefir was significantly different on days 6 and 9, whereas pH got decreased. The lactic acid was formed too much, causing the pH kefir getting down. On the 15th day, pH got increased again (4.13 to 4.26). The declined in lactic acid bacteria population and increased yeast activity that changed lactose to alcohol affected the stability of pH. This result was not different from other studies, which stated pH decreased during the first 7 days, then pH constant until day 14[27-28].

4.7 Viscosity
The viscosity of goat milk kefir fortified with vitamin B12 and vitamin D3 did not differ during storage (p > 0.05). The viscosity of kefir ranges from 0.0393-0.0544 cm²/s. The results of this study differ from previous studies, whereas there was a decreased in viscosity during 14 days storage[30]. Viscosity was affected by lactic acid bacteria, total solids, fats, and proteins which was contained in kefir[31]. Viscosity of kefir increased during a 24-hour fermentation process due to the exopolysaccharide or kefiran. Kefiran is the main polysaccharide produced by the microorganisms found in kefir and its biosynthesis is generally ascribed to Lactobacillus kefir or Lactobacillus kefiranofaciens. Lactic acid bacteria are responsible for the production of extracellular polysaccharides which contribute to a significant degree to the texture of the fermented dairy products in which they are present. Variety of lactic acid bacteria namely Lactobacillus, Streptococcus, Lactococcus, and Leuconostoc. S. tomophilus which interacted with milk proteins which caused increasing viscosity. Furthermore, the reduction of the viscosity can be attributed to a degradation of the protein structure caused by the proteolytic action of microorganisms[17][34].

5. Conclusion
Vitamin B12, protein, fat, and viscosity of goat milk kefir vitamin B12 and D3 fortified stable for 15 days storage. The pH value of kefir followed the standard range of pH kefir, ie 4.2-4.6. This shows that kefir is still worth consuming. However, there was a decrease in vitamin D3 and total lactic acid bacteria on the 15th day. Although total lactic acid bacteria decreased, but still meet the standard of Codex Alimentarius, which is 10^7 cfu/mL.
6. **Acknowledgement**

The author would like to thank Faculty of Medicine fiscal year 2016 for funding this research.

**References**

[1] Folli F, Corradi D, Fanti P, Davalli A, Paez A, Giaccari A, and et al 2011 Curr Diabetes Rev 7 313–24
[2] Badan Penelitian dan Pengembangan Kementerian Kesehatan RI 2013 Riset Kesehatan Dasar. p xvi
[3] Gandhe MB, Jain K, Gandhe SM 2013 J Clin Diagn Res 7 2438
[4] Ho M, Halim JH, Gow ML, El-Haddad N, Marzuli T, and Baur LA 2006 Arch Pediatr Adolesc Med 160 933–6
[5] Allen L, Benoist B, Dary O, Hurrell R, editor 2006 WHO, Food and Agricultural Organization of the United Nation 126–30.
[6] Farnworth ER 2005 Food Science and Technology Bulletin: Functional Foods 2 1–17
[7] Urdaneta E, Barrenetxe J, Aranguren P, Irigoyen A, Marzo F, and Ibáñez FC 2007 Nutr Res 27 653–8
[8] Zenebe T, Ahmed N, Kabeta T, Kebede G, Medicine V, Box PO 2014 Nutr Res 3 30–9
[9] Grossmann RE, Tangpricha V 2011 Mol Nutr Food Res 54 1055–61
[10] Stanton C, Ross RP, Fitzgerald GF, dan Sinderen DV 2005 Curr Opin Biotechnol 16 198–203
[11] Simova E, Beshkova D, Angelov A, Hristozova TS, Frengova G, and Spasov G 2002 J Food Microbiol Biotechnol 19 537–42
[12] Folli F, Corradi D, Fanti P, Davalli A, Paez A, Giaccari A, and et al 2011 Curr Diabetes Rev 7 313–24
[13] Badan Penelitian dan Pengembangan Kementerian Kesehatan RI 2013 Riset Kesehatan Dasar. p xvi
[4] Ho M, Halim JH, Gow ML, El-Haddad N, Marzuli T, and Baur LA 2006 Arch Pediatr Adolesc Med 160 933–6
[5] Allen L, Benoist B, Dary O, Hurrell R, editor 2006 WHO, Food and Agricultural Organization of the United Nation 126–30.
[6] Farnworth ER 2005 Food Science and Technology Bulletin: Functional Foods 2 1–17
[7] Urdaneta E, Barrenetxe J, Aranguren P, Irigoyen A, Marzo F, and Ibáñez FC 2007 Nutr Res 27 653–8
[8] Zenebe T, Ahmed N, Kabeta T, Kebede G, Medicine V, Box PO 2014 Nutr Res 3 30–9
[9] Grossmann RE, Tangpricha V 2011 Mol Nutr Food Res 54 1055–61
[10] Stanton C, Ross RP, Fitzgerald GF, dan Sinderen DV 2005 Curr Opin Biotechnol 16 198–203
[11] Simova E, Beshkova D, Angelov A, Hristozova TS, Frengova G, and Spasov G 2002 J Food Microbiol Biotechnol 19 537–42
[12] Folli F, Corradi D, Fanti P, Davalli A, Paez A, Giaccari A, and et al 2011 Curr Diabetes Rev 7 313–24
[13] Badan Penelitian dan Pengembangan Kementerian Kesehatan RI 2013 Riset Kesehatan Dasar. p xvi
[4] Ho M, Halim JH, Gow ML, El-Haddad N, Marzuli T, and Baur LA 2006 Arch Pediatr Adolesc Med 160 933–6
[5] Allen L, Benoist B, Dary O, Hurrell R, editor 2006 WHO, Food and Agricultural Organization of the United Nation 126–30.
[6] Farnworth ER 2005 Food Science and Technology Bulletin: Functional Foods 2 1–17
[7] Urdaneta E, Barrenetxe J, Aranguren P, Irigoyen A, Marzo F, and Ibáñez FC 2007 Nutr Res 27 653–8
[8] Zenebe T, Ahmed N, Kabeta T, Kebede G, Medicine V, Box PO 2014 Nutr Res 3 30–9
[9] Grossmann RE, Tangpricha V 2011 Mol Nutr Food Res 54 1055–61
[10] Stanton C, Ross RP, Fitzgerald GF, dan Sinderen DV 2005 Curr Opin Biotechnol 16 198–203
[11] Simova E, Beshkova D, Angelov A, Hristozova TS, Frengova G, and Spasov G 2002 J Food Microbiol Biotechnol 19 537–42
[12] Folli F, Corradi D, Fanti P, Davalli A, Paez A, Giaccari A, and et al 2011 Curr Diabetes Rev 7 313–24
[13] Badan Penelitian dan Pengembangan Kementerian Kesehatan RI 2013 Riset Kesehatan Dasar. p xvi
[14] Badan Penelitian dan Pengembangan Kementerian Kesehatan RI 2013 Riset Kesehatan Dasar. p xvi
[15] Simova E, Beshkova D, Angelov A, Hristozova TS, Frengova G, and Spasov G 2002 J Food Microbiol Biotechnol 19 537–42
[12] Folli F, Corradi D, Fanti P, Davalli A, Paez A, Giaccari A, and et al 2011 Curr Diabetes Rev 7 313–24
[13] Badan Penelitian dan Pengembangan Kementerian Kesehatan RI 2013 Riset Kesehatan Dasar. p xvi
[14] Badan Penelitian dan Pengembangan Kementerian Kesehatan RI 2013 Riset Kesehatan Dasar. p xvi
[15] Simova E, Beshkova D, Angelov A, Hristozova TS, Frengova G, and Spasov G 2002 J Food Microbiol Biotechnol 19 537–42
[12] Folli F, Corradi D, Fanti P, Davalli A, Paez A, Giaccari A, and et al 2011 Curr Diabetes Rev 7 313–24
[13] Badan Penelitian dan Pengembangan Kementerian Kesehatan RI 2013 Riset Kesehatan Dasar. p xvi
[14] Badan Penelitian dan Pengembangan Kementerian Kesehatan RI 2013 Riset Kesehatan Dasar. p xvi
[15] Simova E, Beshkova D, Angelov A, Hristozova TS, Frengova G, and Spasov G 2002 J Food Microbiol Biotechnol 19 537–42
[12] Folli F, Corradi D, Fanti P, Davalli A, Paez A, Giaccari A, and et al 2011 Curr Diabetes Rev 7 313–24
[13] Badan Penelitian dan Pengembangan Kementerian Kesehatan RI 2013 Riset Kesehatan Dasar. p xvi
[14] Badan Penelitian dan Pengembangan Kementerian Kesehatan RI 2013 Riset Kesehatan Dasar. p xvi
[15] Simova E, Beshkova D, Angelov A, Hristozova TS, Frengova G, and Spasov G 2002 J Food Microbiol Biotechnol 19 537–42
[12] Folli F, Corradi D, Fanti P, Davalli A, Paez A, Giaccari A, and et al 2011 Curr Diabetes Rev 7 313–24
[13] Badan Penelitian dan Pengembangan Kementerian Kesehatan RI 2013 Riset Kesehatan Dasar. p xvi
[14] Badan Penelitian dan Pengembangan Kementerian Kesehatan RI 2013 Riset Kesehatan Dasar. p xvi
[15] Simova E, Beshkova D, Angelov A, Hristozova TS, Frengova G, and Spasov G 2002 J Food Microbiol Biotechnol 19 537–42
[12] Folli F, Corradi D, Fanti P, Davalli A, Paez A, Giaccari A, and et al 2011 Curr Diabetes Rev 7 313–24
[13] Badan Penelitian dan Pengembangan Kementerian Kesehatan RI 2013 Riset Kesehatan Dasar. p xvi
[14] Badan Penelitian dan Pengembangan Kementerian Kesehatan RI 2013 Riset Kesehatan Dasar. p xvi
[15] Simova E, Beshkova D, Angelov A, Hristozova TS, Frengova G, and Spasov G 2002 J Food Microbiol Biotechnol 19 537–42
[12] Folli F, Corradi D, Fanti P, Davalli A, Paez A, Giaccari A, and et al 2011 Curr Diabetes Rev 7 313–24
[13] Badan Penelitian dan Pengembangan Kementerian Kesehatan RI 2013 Riset Kesehatan Dasar. p xvi
[14] Badan Penelitian dan Pengembangan Kementerian Kesehatan RI 2013 Riset Kesehatan Dasar. p xvi
[15] Simova E, Beshkova D, Angelov A, Hristozova TS, Frengova G, and Spasov G 2002 J Food Microbiol Biotechnol 19 537–42