Journal of
Food and Pharmaceutical Sciences

*Original Article*

# The Effect of Milk Kefir with Additional Sorghum Flour on Weight, Appetite, and Abdominal Fat in Mice Obesity Induced

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Received: 6 September 2022; Revised: 18 December 2022; Accepted: 20 December 2022; Published: 31 December 2022

**Abstract:** In obese patients, one way to prevent obesity with the right method is to modulate the intestinal microflora. The purpose of this study was to identify and analyze the differences in administration of milk kefir with sorghum flour on body weight, appetite, and abdominal fat in mice (*Mus musculus*) induced obesity.This type of research is a true experimental in vivo using a randomized controlled design pretest – posttest control group design. The experimental animals used were 34 healthy mice (*Mus musculus*). Data analysis with normal distribution was tested by One Way Anova, otherwise using the Kruskal Wallis test. Meanwhile, categorical data was tested Univariate. The results showed that in the third treatment (T3) the mice were given milk kefir with sorghum flour and high fat diet feed for 8 weeks or 56 days there was a difference in weight loss as measured by weight gain ($p<0.05$), then there wasn't difference in appetite ($p>0.05$), while there was a difference in abdominal fat compared to the control group. So for those who are obese, milk kefir products with sorghum can be used as a daily drink to help lose weight and fat in the abdomen.

**Keywords:** obesity; milk kefir; sorghum, high fat diet

## 1. INTRODUCTION

The prevalence of obesity or being overweight is increasing in many parts of the country. Indonesia is one country that is experiencing the Triple Burden of Malnutrition problem (obesity). There are more than 340 million children and adolescents ranging in age from 5 to 9 years, and in adults aged 18 years and over worldwide in 2016 more than 1.9 billion were overweight [1]. Someone who is overweight can lead to possibility of complications of various diseases, such as diabetes mellitus, osteoarthritis, cancer, kidney failure, heart disease, and asthma which become a heavy economic burden for the community [2]. Bad lifestyle and eating patterns, climate change, and stress can disrupt the balance of microflora in the digestive tract resulting in less beneficial microbes dominating [3].

Intestinal microbiota dysbiosis is responsible for certain diseases in the host, such as obesity, cancer, and inflammatory bowel disease. Therefore, modulating gut microflora is an appropriate method to prevent obesity [4]. Consuming probiotic (lactobacilli) in the various bacterial strains are

capable to confer beneficial effects in the prevention of other related metabolic disorders, including obesity [5]. This is due to the effect of probiotics on the gut microbiota which is a major driver of obesity and inflammation. One of the foods to treat and prevent people with obesity is functional food products. Functional food products are processed food or natural food that have contents active biological components, known or unknown, don't have toxic effects, has been clinically proven and documented to give health benefits for the management, prevention, and treatment of some disease [6]. The components of functional food are prebiotics, probiotics, although a combination of both prebiotics and probiotics known as synbiotic food [7].

Milk kefir grains contain probiotics with various cultures of lactic acid bacteria from the genera *Lactococci*, *Lactobacilli*, and yeast *Candida kefir*, *Saccharomyces cerevisiae* and *Kluyveromyces lactis*. Lactobacillus has many subspecies, some of which have anti-obesity effects. Consuming kefir regularly can accelerate the body's metabolic system during the fat burning process which results in weight loss [8].

Sources of food containing prebiotics come from tubers, vegetables, and fruits. One of the most potential sources of prebiotic is prebiotic food from the carbohydrate group [9]. Sorghum contains resistant starch and oligosaccharides that cannot be digested by the body. Dietary fiber, resistant starch, and oligosaccharides found in sorghum are processed by probiotic bacteria in the large intestine for growth and reproduction. So, synbiotic drinks are the right drinks to prevent obesity. Based on this background, the purpose of this study was to identify the effect of giving milk kefir with sorghum flour on body weight, appetite, and abdominal fat in mice (*Mus musculus*) induced by obesity.

## 2. MATERIALS AND METHODS

### *2.1. Making Milk Kefir with Sorghum*

The preparation in the making milk kefir with sorghum are grains, sterilized milk, and sorghum. The total of 25 gr for these grains were cultivated in 200 ml sterillized milk at 40ºC. Then, add sorghum flour in to sterilized milk. After that, leave for 24 hours for fermentation process, then store in the refrigerator.

### *2.2. Making High Fat Diet Feed*

The preparation in making high fat diet feed are beef fat, goat fat, margarine, egg, water, HI – PROVITE Medicated Voer 594 chicken feed, catfish pellets, and flour. Mix the egg yolk with beef oil until a corpus emulsion is formed in the base, then add water up to 100 ml into the emulsion corpus, then stir quickly until a smooth and homogeneous emulsion is formed.

### *2.3. Treatment and Sampling*

This in vivo test uses mice (Mus musculus) aged 2-3 months that weight ± 21-25 grams. This testing process was carried out for 8 weeks with the oral feeding method which was carried out at the Animal Laboratory of the Faculty of Pharmacy, Airlangga University. Variations of treatment given was that the first treatment mice (T1) were given normal feed (N), the second treatment (T2) were given High Fat Diet (HFD), the third treatment (T3) were given High Fat Diet and Milk Kefir with Sorghum flour (HFD + KS). The provision of milk kefir with sorghum flour is calculated with an acceptable serving size or Acceptable Daily Intake (ADI) which was previously converted to the weight of mice, so the result is 0.7 g/weight of mice.

*2.4. Weight Measurement*

The test was conducted to determine the effect of giving milk kefir with sorghum flour on body weight. The value of mice weight is obtained from measuring body weight in mice using special scales for experimental mice (Triple Beam Balance, OHAUS) or digital food scales by calculating the weight gain of mice = final weight – initial body weight.

*2.5. Appetite Measurement*

The test was conducted to determine the effect of giving milk kefir with sorghum flour on appetite. If you want to know the feeding intake of standard feed or a high-fat diet, weighing the remaining feed every day, after that it can be used to find out the average amount of feed consumed,

JPD (amount of feed consumed) = The amount of feed given - The amount of feed residue.

*2.6. Fat Abdominal Measurement*

The test was conducted to determine the effect of giving milk kefir with sorghum flour on abdominal fat. It was seen from the mice was given feed every day, while abdominal fat was measured and observed after surgery. The distribution of abdominal fat is calculated through the percentage of dietary fat intake given in grams per day. Then, the spread of abdominal fat is categorized according to the weighing results, that are normal, quite normal, and excessive.

*2.7. Processing and Data Analysis*

Data analysis used a significance level of <0.05. Differences in body weight were analyzed using One-way ANOVA parametric statistical test and then Tukey's Post Hoc Test resulted in $p<0.05$. This indicates that there is a significant difference between the groups tested. Meanwhile, on the distribution of abnormal data is food intake, the Kruskal Wallis non-parametric statistical test is carried out. Meanwhile, the data analysis of the results of the study of differences in abdominal fat was carried out by classifying categorical data and then tested with univariate with categorical data. Then, the difference in abdominal fat data is presented visually which will be described. All statistical analysis will be carried out with the spss application.

## 3. RESULTS AND DISCUSSION

*3.1. Weight*

Based on the one way ANOVA test in Table 1 after 8 weeks, it can be informed that there is a difference in the effect of giving milk kefir with sorghum flour and a high fat diet on the Weight Gain (WG) of mice. This can be seen on the Weight Gain (WG) of mice significant value ($p = 0,00$). Meanwhile, the Initial Body Weight (IBW) has a significant value ($p = 0,906$) and the Final Body Weight (FBW) has a significant value ($p = 0,306$). The results of the study informed that the treatment when giving a milk kefir with sorghum flour and high fat diet (T3) can reduce body weight or prevent obesity in mice.

Milk kefir with sorghum flour can cause weight loss because kefir has the potential as an anti-obesity food that can accelerate the body's metabolic system during the fat burning process which results in weight loss. This is because kefir contains MUFA (Monounsaturated Fatty Acids) 75.6% or unsaturated fatty acids and SFA (Saturated Fatty Acids) 22.8% or saturated fatty acids. In addition to the content of MUFA unsaturated fatty acids, kefir has a high oleic acid content of 18-19.8% [10]. Oleic acid is a fat that is beneficial for the body to increase HDL cholesterol and reduce LDL cholesterol

levels which can prevent and reduce the risk of heart disease [11]. So, the role of kefir in obesity has the effect of accelerating fat metabolism.

**Table 1.** Mean Change in Body Weight Before and After First Treatment (T1) Normal Feed (N), Second (T2) High Fat Diet Feed (HFD), and Third (T3) High Fat Diet Feed With Milk Kefir With Sorghum Flour (HFD + KS).

| Group | N | IBW | FBW | WG | IBW | FBW | WG |
|---|---|---|---|---|---|---|---|
| | | X̄ ± SD | X̄ ± SD | X̄ ± SD | | | |
| T1 | 11 | 23.18 ± 1.53 | 26.66 ± 1.77 | 3.48 ± 0.23 | | | |
| T2 | 11 | 23.09 ± 1.45 | 27.71 ± 1.74 | 4.62 ± 0.29 | 0.906 | 0.306 | 0.00* |
| T3 | 12 | 22.92 ± 1.38 | 26.81 ± 1.61 | 3.99 ± 0.23 | | | |

The addition of sorghum to kefir, according to Shen (2015) starch contained in sorghum can reduce overweight and obesity by modulating adipokine levels and increasing the number of lactobacillus and bifidobacterium in sprague-dawley rats [12]. Then on sorghum flour there is also a study conducted by Arbex (2018) showing that sorghum can affect weight loss and food intake in obese rats induced by a high-fat diet. This is because sorghum contains good nutrition, such as high protein of 10.6 g [13]. Then, sorghum also contains gluten and a lower glycemic index (GI) [14]. Glycemic index is an important tool used in planning diet someone who have diabetes and in weight loss programs.Lower glycemic index foods have a process slow digestion and absorption, so that the body produce a more gradual rise in insulin levels to handle blood sugar are increasingly [15].In addition, the content of sorghum flour has a fiber of 8.83% which includes soluble fiber of 2.39% and water insoluble fiber of 6.44%. One of them is insoluble fiber which can help gel formation to slow gastric emptying, slow down transit in the small intestine, and reduce glucose diffusion, resulting in a decrease in postprandial blood glucose. This system is carried out by stimulating the formation of insulin and short chain fatty acids through fermentation so that there is a decrease in blood glucose by accelerating the release of incretin hormones produced in the intestine [16].

*3.2. Appetite*

Based on the p-value in Table 2, the Kruskal Wallis test can be informed that there is an effect on each treatment given normal feed, high fat diet feed, and milk kefir with sorghum flour and high fat diet feed on the total feed provided, feed consumed, and leftover feed provided. Feed given to mice with a significance value ($p>0.05$). The results of the study of appetite in each treatment there was no significant difference because all mice had the same appetite. However, in the treatment without milk kefir with sorghum flour, it ran out faster. While the treatment given kefir with sorghum had a longer feeding time to spend the feed given. So it can be concluded that when drinking milk kefir with sorghum flour can not reduce appetite in mice, it only gives a feeling of satiety for a while.

**Table 2.** Average of First Treatment (T1), Normal Feed (N), Second (T2) High Fat Diet Feed (HFD), and Third (T3) High Fat Diet Feed With Milk Kefir With Sorghum Flour (HFD + KS) Against Total Feed Provided (TFP), Feed Consumed (FC), and Leftover Feed Provided (LFP).

| Group | N | TFP | FC | LFP | TFP | FC | LFP |
|---|---|---|---|---|---|---|---|
| | | X̄ ± SD | X̄ ± SD | X̄ ± SD | *Pvalue* | | |
| T1 | 11 | 84.5 ± 0.00 | 84.5 ± 0.23 | 84.5 ± 0.24 | | | |
| T2 | 11 | 84.5 ± 0.00 | 84.5 ± 0.23 | 84.5 ± 0.24 | 1.00 | 1.00 | 1.00 |
| T3 | 12 | 84.5 ± 0.00 | 84.5 ± 0.23 | 84.5 ± 0.24 | | | |

Milk kefir with sorghum is a probiotic or synbiotic drink that provides a short feeling of fullness. This is because appetite can be affected by several factors, one of which is the work of the hormone leptin in the body. The incidence of obesity or overweight experiencing leptin deficiency that can occur in experimental animals and humans.Leptin is a hormone produced by adipose tissue, associated with the work of the hypothalamus, plays a role in weight regulation, appetite (hunger and satiety), and neuroendocrine function [17]. However, kefir contains good probiotics which are known to increase the population of Lactobacillus, Lactococcus and Candida at the molecular level showing a strong positive relationship with PPAR-$\alpha$ gene expression in both liver and adipose tissue. Sorghum has a high fiber content. Insoluble fiber will be fermented in the large intestine to help the growth of intestinal microflora, which is a class of probiotic species [18]. Prebiotics are nutritional supplements that function to increase the number of beneficial bacteria in the gut. The synbiotic effect is obtained when a product contains probiotics and prebiotics. These mutually beneficial effects, such as regulating appetite (leptin and ghrelin), increasing nutrient utilization, and nutrient metabolism mechanisms, such as control of blood sugar, cholesterol, and amino acids [19].

*3.3. Abdominal Fat*

In Table 3, the results of the 8 week study based on the Univariate test can be informed that there is a relationship between treatment groups on the distribution of abdominal fat. The difference in this study can be seen after the surgery in Figure 1. The distribution of abdominal fat in mice which can be seen visually is a difference in each treatment, then the distribution of abdominal fat is calculated through the percentage of dietary fat intake given in grams per day. The results of the data on the difference in abdominal fat of mice fed standard or normal diets did affect the amount of fat spread ± 0.15 - 0.2 g around the abdomen because the dose of normal feed, especially fat had been adjusted, T2 mice fed high fat diet affect the amount of fat spread around the abdomen ± 4 - 4.1 g, T3 mice fed a high fat diet and milk kefir with sorghum flour affect the amount of fat spread around the abdomen ± 0.32 - 0.39 g, however less than the treatment with high fat diet. So, consuming milk kefir with sorghum can reduce abdominal fat.

**Table 3.** Distribution of Abdominal Fat by Level of Distribution of Abdominal Fat

| Group | Degree of Spread of Abdominal Fat | N | Precentage (%) |
|---|---|---|---|
| T1 | Normal | 11 | 32.4 |
| T2 | Quite Normal | 12 | 35.3 |
| T3 | Excessive. | 11 | 32.4 |
| Entire | | 34 | 100 |

In milk kefir, it is known that the increase in the population of Lactobacillus, Lactococcus and Candida at the molecular level shows a strong positive relationship with the expression of the PPAR-$\alpha$ gene in both liver and adipose tissue. PPAR-$\alpha$ activation affects fatty acid uptake, utilization, and catabolism by upregulation of genes involved in fatty acid transport, lipid binding and activation, and peroxisomal and mitochondrial fatty acid beta-oxidation, resulting in decreased fat accumulation. PPAR-$\alpha$ is an upstream regulator of AOX. AOX is significantly upregulated in the liver and adipose tissue.

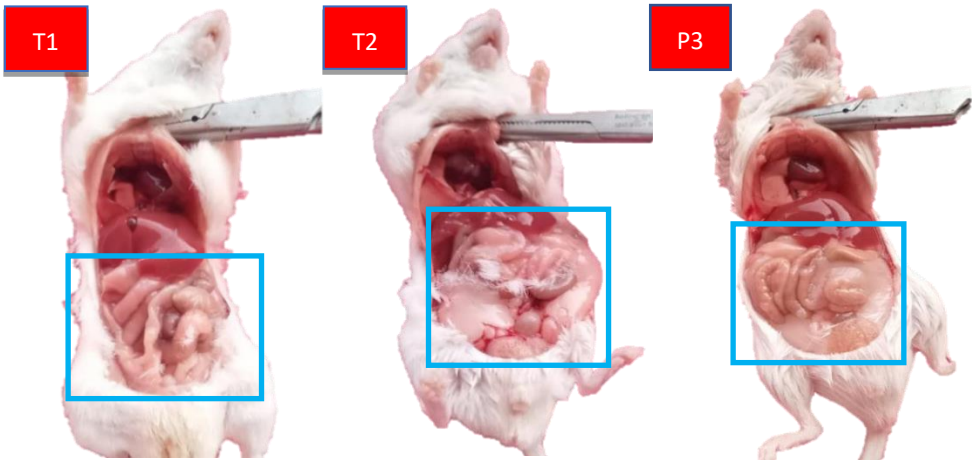


**Figure 1.** Differences in abdominal fat in the First Treatment (T1) Normal Feed (N), Second (T2), High Fat Diet Feed (HFD), and Third (T3) High Fat Diet Feed and Milk Kefir With Sorghum Flour (HFD + KS ) observations through visuals are then taken with a camera. The spread of fat can be seen around the abdominal organs

This suggests that the upregulation of fatty acid oxidation in both organs is caused by bacteria and yeast introduced by kefir intake, and their interaction with the host results in the anti-obesity and anti-NAFDL effect of kefir. Kefir consumption significantly reduced plasma concentrations of the proinflammatory cytokine IL-6 and downregulated MCT1 mRNA expression. This means that consuming fermented milk results in a significant reduction in visceral and subcutaneous fat which may be associated with fat loss in overweight subject [20]. Then, the addition of sorghum flour in kefir affects adipose tissue in the incidence of obesity by inhibiting the increase in proinflammatory leptin so that it increases the expression of adipokines and cytokines [21]. So it can be concluded that the treatment of milk kefir with sorghum flour can reduce fat in mice that given a high fat diet every day. Moreover, bioactive components in there that could reduce cholesterol levels such as dietary fiber and lactic acid bacteria [22].

## 4. CONCLUSION

Based on the results of this study, it can be concluded that the administration of a high fat diet of milk kefir with sorghum flour for 8 weeks has an effect on body weight which can reduce weight or prevent obesity in mice even though the mice are fed a high fat diet. Then, the appearance of abdominal fat in mice fed with kefir milk with sorghum has a fairly normal distribution of belly fat and fat weight compared to mice fed a high-fat diet. But, there was no difference on appetite in mice, it means that each group has the same appetite.

**Conflicts of interest:** The authors declare no conflict of interest.

## References

1. World Health Organization, 2016. Obesity, [online], (https://www.who.int/health-topics/obesity), accessed 21 Agustus 2021.
2. Cao, S.Y., Zhao, C.N., Xu, X.Y., Tang, G.Y., Corke, H., Gan, R.Y., & Li, H.B. 2019. Dietary Plants, Gut Microbiota, and Obesity : Effect and Mechanisms. Trends in Food Science dan Technology, 92:194-204. DOI. 10.1016/j.tifs.2019.08.004
3. Aryanta, I.W.R., 2021, Kefir dan Manfaatnya Bagi Kesehatan. E-Jurnal Widya Kesehatan, 3(1):35-38.

4. Kim, D.H., Kim, H., Jeong, D., Kang, I.B., Chon, J.W., Kim, H.S., Song, K.Y., and Seo, K.H. 2017. Kefir Alleviates Obesity And Hepatic Steatosis In High Fat Died Fed Mice by Modulation of Gut Microbiota and Mycobiota : Targeted and Untargeted Community Analysis With Correlation of Biomarkers. The Journal of Nutritional Biochemistry, 44:1-35. DOI.10.1016/j.jnutbio.2017.02.014
5. Pothuraju, R., Yenuganti, V.R., Hussain, S.A., and Sharma, M. 2017. Chapter 29. Fermented Milk in Protection Against Inflammatory Mechanisms in Obesity, in Immunity and Inflammation in Health and Disease, edited by Shampa, C., Wolfgang, J., and Bagchi, D., Academic Press.pp 389-401.
6. Barus, C.P.U., Julianti, E., and Yusraini, E. 2019. The Effect of Addition of Pridot Leaf Extract and Juice of Red Watermelon Albedo on Carbonated Coconut Drink of Blood Glucose Levels of Mice. Journal of Food and Pharmaceutical Sciences. 7(1) : 25-33.
7. Sukarminah E., Mardawati, E., Wulandari E., Cahyana Y., and Ningsih B.D. 2017. Pengaruh Konsentrasi Tepung Sorgum (*Sorghum bicolor L. Moench)* Terhadap Beberapa Karakteristik Minuman Sinbiotik. Asian Journal of Environment, History, and Heritage, 1(2):1-11.
8. Khuswatun, K.S., Susanti, S., and Legowo, A.M. 2020. Karakteristik Es Krim Kefir Puree Buah Naga Merah Sebagai Pangan Fungsional Antiobesitas. Journal of Food Technology and Nutrition : Jurnal Teknologi Pangan dan Gizi. 19(2):53-62, DOI. 10.33508/jtpg.v19i2.2749.
9. Masrikhiyah, R., Prasetya, H.W., Ubaedillah, U., Balfas, R. F., and Yulianingsih, S. 2020. Peningkatan Pengetahuan Mengenai Manfaat Pangan Probiotik Dan Prebiotik Bagi Kesehatan. Jurnal Abdi Masyarakat UMUS (JAMU), 1(1):42-46. DOI. 10.46772/jamu.v1i01.316.
10. Alves, E., Ntungwe, E.N., Gregorio, J., Rodrigues, L. M., Leite, C. P., Caleja, C., Pereira, E., Barros, L., and Rijo, P. 2021. Characterization of Kefir Produced in Household Conditions : Physichemical and Nutritional Profile, and Storage Stability. Foods, 10(5):2-16. DOI: 10.3390/foods10051057.
11. Aisyah, N.F., Aisyah, N., Kusuma, T.S., and Widyanto, R. M. 2019. Profil Asam Lemak Jenuh dan Tak Jenuh Serta Kandungan Kolesterol Nugget Daging Kelinci New Zealand White (*Oryctolagus cuniculus*). Jurnal Al-Azhar Indonesia Seri Sains Dan Teknologi. 5(2):92 – 100. DOI: 10.36722/sst.v5i2.356.
12. Shen, R.L., Zhang, W.L., Dong, J.L., Ren, G.X., and Chen, M. 2015. Sorghum Resistant Starch Reduces Adiposity in High-Fat-Diet-Induced Overweight and Obese Rats Via Mechanisms Involving Adipokines and Intestinal Flora. Food and Agricultural Immunology, 26(1):120-130. DOI. 10.1080/09540105.2013.876976.
13. Arbex, P.M. Moreira, M.E.C., Toledo, R.C.L., Cardoso, L.M., Santana, H.M.P., Benjamin, L.A., Licursi, L., Carvalho, C.W.P., Queiroz, V.A.V. and Martino, H.S.D. 2018. Extruded Sorghum Flour (*Sorghum Bicolor L.)* Modulate Adiposity And Inflammation In High Fat Diet-Induced Obese Rats". Journal of Functional Foods, 42:346-355. DOI. 10.1016/j.jff.2018.01.010.
14. Sumarmo. 2013. Sorgum Inovasi Teknologi dan Pengembangan. Jakarta : IAARD Press.
15. Ijarotimi, O.S., Fagbemi, T.N., and Osundahunsi, O.F. 2015. Determination of Nutrient Compositions, Glyceamic Index and Ant-diabetic Potentials of Multi-plant Based Functional Foods in Rats. Journal of Food and Pharmaceutical Sciences. 3:55-63.
16. Dewi, A.C., Widyastuti, N., and Probosari, E. 2020. Pengaruh Pemberian Tepung Sorgum (Sorghum Bicolor L. Moench) Terhadap Kadar Glukosa Darah Puasa Tikus Diabetes". Journal Of Nutrititon College, 9(1):63-70.
17. Farooqi, L.S., Bullmore, E., Keogh, J., Gillard J., Orahilly, S., and Fletcher, P.C. 2014. Leptin Regulates Striatal Regions and Human Eating Behavior. Science, 317(7):1355. DOI. 10.1126/science.1144599.

18. Ambasari, I., Endrasari, R., and Hidayah, R. 2020. Kandungan Nutrisi Dan Kualitas Sensoris Produk Minuman Sereal Sarapan Berbasis Flakes Jagung, Jali, Dan Sorgum. Jurnal Penelitian Pascapanen Pertanian,17(2):108-116.
19. Kusumo, P.D. 2010. Potensi Probiotik Dalam Mekanisme Sistem Imunitas. Majalah Kedokteran FK UKI, 4:184-193. DOI: 10.33541/mkvol34iss2pp60.
20. Kadooka, Y., Sato, M., Imaizumi, K., Ogawa, A., Ikuyama, K., Akai, Y., Okano, M., Kagoshima, M., and Tsuchida. 2010. Regulation Of Abdominal Adiposity By Probiotics (Lactobacillus Gasseri SBT2055) In Adults With Obese Tendencies In A Randomized Controlled Trial. European Journal of Clinical Nutrition, 64:636-643. DOI: 10.1038/ejcn.2010.19.
21. Rahayu, R.L., Mubarok, A.Z., and Istianah, N. 2021. Hipertensi, Diabetes Melitus, Dan Obesitas Sebagai Faktor Komorbiditas Utama Terhadap Mortalitas Pasien Covid-19: Sebuah Studi Literatur. JIMKI : Jurnal Ilmiah Mahasiswa Kedokteran Indonesia, 9(1):90-97. DOI: 10.21776/ub.jpa.2021.009.02.3.
22. Lestari, L. A., Amirina, Q. S., Anindyah, D. S., Nisa, A., Huda, A. N., Indrianingrum, W., Prasetyastuti, and Nurliyani. 2020. Characteristics of Purple Sweet Potato Yogurt and Its Effect on Lipid Profiles of Sprague Dawley Rats Fed with High Fat Diet. Journal of Food and Pharmaceutical Sciences. 8(2) : 240-251.