



Functional cookies made from black rice for oxidative stress control in overage and obesity adolescents

Pemberian cookies fungsional beras hitam untuk pengendalian stres oksidatif pada remaja yang mengalami kegemukan dan obesitas

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Abstract

Health problems in Indonesia generally occur because of the unhealthy lifestyles of adolescents or sedentary lifestyles. The most common cases are health problems, such as obesity and overweight. This study aimed to measure the effect of providing functional cookies on controlling oxidative stress in obese adolescents, determine the compliance of cookie consumption by subjects, and conduct consumer tests. The study used an experimental design conducted from August to September 2022 at SMP Negeri 7 Cirebon City. The participants were 24 adolescents aged 13-15 years. Data were collected using questionnaires and analyzed for malondialdehyde (MDA) and Superoxide Dismutase (SOD) levels in the blood serum. Data analysis was performed using the Mann-Whitney U test and Pearson correlation test at 95% CI. The results showed that the provision of functional cookies significantly ($p < 0,05$) reduced serum malondialdehyde (MDA) levels in overweight and normal adolescents ($p = 0,017$). Superoxide dismutase (SOD) enzyme activity had no effect on overweight/obese and normal adolescents after providing functional cookies. In conclusion, providing functional cookies can reduce MDA levels in obese and normal adolescents ($p = 0,017$) but had no effect on SOD enzyme activity. The adherence to cookie consumption and consumer test results support its potential in controlling oxidative stress in adolescents.

Keywords: Adolescents, Cookies, MDA, Obesity, SOD

Abstrak

Gangguan kesehatan di Indonesia umumnya terjadi karena pola hidup remaja tidak sehat atau *sedentary life*. Kasus yang paling banyak terjadi adalah gangguan kesehatan seperti obesitas (*obesity*) dan kelebihan berat badan (*overweight*). Tujuan penelitian adalah untuk mengukur pengaruh pemberian cookies fungsional dalam mengendalikan stres oksidatif pada remaja yang mengalami obesitas, mengetahui kepatuhan konsumsi cookies oleh subyek, dan melakukan uji konsumen. Penelitian menggunakan desain eksperimen, telah dilakukan pada bulan Agustus-September 2022 di SMP Negeri 7 Kota Cirebon. Subjek sebanyak 24 remaja berusia 13-15 tahun. Pengumpulan data menggunakan kuesioner dan analisis kadar Malondialdehid (MDA) dan Superoksida Dismutase (SOD) serum darah subjek. Analisis data menggunakan uji Mann-Whitney dan uji korelasi Pearson pada CI 95%. Hasil, ditemukan bahwa pemberian produk cookies fungsional secara signifikan ($p < 0,05$) menurunkan kadar serum malondialdehida (MDA) pada remaja yang mengalami kegemukan maupun remaja yang normal dengan nilai ($p = 0,017$). Aktivitas enzim superoksida dismutase (SOD) tidak berpengaruh setelah pemberian produk cookies fungsional pada remaja kegemukkan/obes maupun yang normal. Kesimpulan, pemberian cookies fungsional dapat menurunkan kadar MDA pada remaja obesitas dan normal ($p = 0,017$), namun tidak berpengaruh

terhadap aktivitas enzim SOD. Kepatuhan konsumsi cookies dan hasil uji konsumen mendukung potensinya dalam mengendalikan stres oksidatif pada remaja.

Kata Kunci: Cookies, MDA, Obesitas, Remaja, SOD

Introduction

Health problems in Indonesia generally occur because of teenagers' unhealthy lifestyles. The most common cases are cases of health problems, such as obesity and overweight. Functional food has had a positive trend as a preventive measure for increasing cases of lifestyle-related diseases (Patil dan Kaur 2018). Functional foods can be conventional foods, modified foods, or synthetic food ingredients with bioactive components (Galanakis, 2021). Food ingredients, such as cereals, are known to contain phytochemicals, such as phenolic compounds, antioxidants, and fiber, which are relatively abundant (Ghasemzadeh et al., 2018; Shao et al., 2018). Phytochemical compounds and antioxidants are known to play a role in preventing chronic diseases, some of which are degenerative, such as diabetes mellitus and obesity (Muscolo et al., 2024).

Adolescence is a period in which growth and development occur rapidly, thus requiring sufficient nutritional intake; this is called the critical period (Lupiana et al., 2022). Peer factors, surrounding environment, and social media significantly influence this condition (Artadini et al., 2022; Lidiawati et al., 2020). In addition, unhealthy eating patterns, sedentary lifestyles, and sleep duration are some of the causes of obesity in adolescents in SMA Negeri 1 Demak (Amrynia and Prameswari 2022).

According to the 2018 Basic Health Research (Risikesdas), there was an increase in the prevalence of central obesity by 4,4% in the population aged ≥ 15 years compared with the previous year's Risikesdas data. The national prevalence of central obesity in 2018 was 31,0%, which was higher than the 26,6% reported in 2013. Obesity prevention can be achieved using various approaches. Some of these are nutrition education (Simbolon et al., 2018), physical activity patterns (Salam, 2010; Wijayanti, 2013), and diet (Wijayanti, 2013). Good food consumption patterns can be an alternative for preventing and treating obesity, including the consumption of functional foods. Antioxidant activity in the body of obese people is usually lower than that in people of normal weight (Dara et. al, 2016). Therefore, it is recommended to increase the consumption of

foods high in fiber, antioxidants, and anthocyanins, such as fruits and vegetables, to help overcome obesity problems and prevent degenerative diseases.

Several mechanisms have been proposed to explain oxidative stress in obesity. Among them is an imbalance between the number of free radicals and antioxidants, which causes oxidative stress (Azizah, 2019). Obesity and oxidative stress can occur during the first two decades of life, and chronic exposure can contribute to the initiation and development of various degenerative diseases. Obesity, combined with low antioxidant intake, worsens oxidative stress. Oxidative stress is exacerbated with increasing age (Vincent et al., 2007; Savini et al., 2013).

Many factors trigger oxidative stress in obesity, including increased blood sugar (hyperglycemia), high blood fat levels (hyperlipidemia), chronic low-grade inflammation, excess leptin hormone (hyperleptinemia), increased muscle activity, and impaired endothelial function (Zulfahmidah et al., 2021). An effective approach to reduce oxidative stress and the potential for various degenerative diseases is to modify the diet. Several studies on diet modification and oxidative stress have reported a tendency to decrease MDA (malondialdehyde) levels (Aleksandrova et al., 2021; Ávila-Escalante et al., 2020).

The most popular rice is white rice because it has a soft texture. However, white rice has disadvantages in terms of health functions compared to other types of rice, such as red and black rice. Red and black rice contain phenolic compounds that are dominated by flavonoids and anthocyanins (Widyawati et al. 2014). According to Widyawati et al. (2014), the highest phenolic content of the three types of rice is found in red rice. Red rice had the highest total flavonoid content, while black rice had the highest anthocyanin content. Phenolic compounds in rice can reduce the risk of health problems such as oxidative stress, diabetes mellitus, and obesity (Muscolo et al. 2024).

A simple method of processing red and black rice is to make flour. The advantages of processing flour from local food include longer shelf life, easier storage, more practical

diversification of processed products, increased value of red and black rice, and reduced use of wheat (Silfia, 2012). Processed flour can be used as the main ingredient in various types of products such as cookies. Cookies or pastries are products that have a long shelf life, so they are easy to store for a long time and are easy to carry around because of their light size and weight due to the drying process. Thus, this study aimed to identify the effect of providing functional cookies on markers of oxidative stress in adolescents who are overweight or obese.

Methods

This study was conducted using a quasi-experimental design from August to September 2022 at SMP Negeri 7, Cirebon City. The samples in this study were cookies made from red and black rice prepared at PT. Choice Plus Makmur, Semarang Regency. The implementation of this study met the Ethical Clearance of the Ethics Commission of the Health Polytechnic of the Ministry of Health of Tasikmalaya, as shown in the EC certificate No. DP.04.03/F.XXVI.20/136/2022.

Research Subjects

This study used two groups of subjects using purposive random sampling: 1) a group of subjects with a body mass index (BMI) of 25 kg/m² or the obesity group and 2) a group of subjects with a BMI of 18,5-24,9 kg/m² or the normal group. Subject selection was also carried out with the following inclusion criteria: subjects who did not consume antioxidant supplements, did not have chronic diseases, and did not have heart, liver, or sleep apnea disorders (temporary cessation of breathing). Subjects who withdrew or were sick during the data collection process were excluded from the study. Subjects who were willing were given an explanation of the study and were asked for consent together with their parents.

Data Retrieval

Data collection in the form of respondent characteristics, including the gender and age of respondents, was carried out through interviews with questionnaires that had previously been tested for validity and reliability. Meanwhile, measurements of waist circumference, percent fat, percent fluid, and percent muscle mass were carried out using a digital body fat scale with an accuracy of 0,1 kg and a capacity of 180 kg. Height was measured using a stadiometer with an accuracy of 0,1 cm and a capacity of 200 cm.

Waist circumference was measured using a waist circumference measuring instrument with an accuracy of 0,1 cm and a capacity of 200 cm.

MDA and SOD data were obtained from the analysis of blood samples from the research subjects. Blood samples were collected in the morning after the subjects had fasted for 10 hours. Blood sampling was performed by laboratory staff at the Pamitran Health Center, Cirebon City. Blood samples were collected using a 5 cc disposable syringe. Blood was placed in an aluminum foil protective tube and immediately centrifuged at the Pamitran Health Center Laboratory. The blood serum was analyzed for MDA and SOD levels at the Physiology Laboratory, Faculty of Medicine, Brawijaya University, Malang, East Java.

Data Processing and Analysis

Respondent characteristic data such as age and gender are processed descriptively. Weight and height data were processed to obtain body mass index (BMI) values: weight (kg)/height (m)².

Data were processed and analyzed using Microsoft Office Excel 2010 and SPSS version 25 data processing programs. Data analysis was performed descriptively and inferentially, at a significance level of 5% ($p < 0,05$). Differences in sex, age with BMI were assessed using an independent t-test. Differences in the mean MDA and SOD levels between the obese and normal groups were assessed using the Mann-Whitney test. The relationship between BMI, LP, and %LT and oxidative stress markers (MDA and SOD) was determined using the Pearson correlation test.

Result and Discussion

Subject Characteristics

The characteristics of the participants are shown in Table 1. There were 24 subjects in this study, namely 12 people in the obese group and 12 people in the normal group.

Table 1. Subject characteristics based on subject groups

Characteristics	Obesity Groups (n=12)	Normal Groups (n=12)
Age (years) ^b	12,93 ± 0,59	12,95 ± 0,45
Body Mass Index (kg/m ²) ^a	3,07 ± 0,47*	0,15 ± 1,25*

Note: *Numbers with different letters in the same row indicate significant differences between treatment groups ($P < 0,05$).

Based on the subject characteristics, the table including age was not significant ($p>0,05$), whereas BMI was significant ($p<0,05$). Obesity is a health condition caused by a long-term imbalance between energy intake and expenditure due to various factors (Yosika et al., 2020). These factors include diet, a family history of obesity, and lifestyle (Irawan, 2013).

Cookie-Tem Product Consumption Compliance

Cookie consumption compliance was determined by the number of cookies consumed per day. The participants were compliant because the average number of cookies consumed each day was more than 95%. The average total compliance to cookies in the obese and normal groups consumed for 21 days was 98,6% in the obese group and 96,2% in the normal group.

The number of cookies consumed by the obese group was higher than that in the normal group. Some respondents did not finish their

cookies because they did not like and were not used to the taste of black rice.

The Effect of Giving Functional Cookies Products on Malondialdehyde (MDA) and Superoxide Dismutase (SOD) Levels

The effect of the functional cookies product intervention on oxidative stress in obese students was identified using serum MDA and SOD biomarkers. Oxidative stress is a condition in which the number of free radicals in the body is higher than the number of endogenous antioxidants (Irawan, 2013).

Changes in Malondialdehyde (MDA) levels can indicate oxidative stress (Yosika et al. 2019). MDA is one of the end products of lipid peroxidation, which occurs when hydroxyl free radicals ($\text{OH}\cdot$) degrade unsaturated fatty acids, resulting in the formation of highly reactive radicals (Nisa, 2017). The mean serum malonaldehyde (MDA) levels of study participants are presented in Table 2.

Table 2. Mean serum MDA levels by subject group

Subject Groups	Serum Malonaldehyde Levels (ng/mL)			p-value
	Before Intervention Average + Deviation	After Intervention Average + Deviation	Average Difference	
Obesity Groups	447,88 $\pm$ 241,77	205,33 $\pm$ 159,96	-242,55	0,017*
Normal Groups	696,09 $\pm$ 437,09	115,31 $\pm$ 58,28	-580,78	

Note: *Numbers with different letters in the same column indicate significant differences between treatment groups ($P < 0,05$).

Table 3. Mean values of SOD enzyme activity based on subject groups

Subject Groups	Superoxide Dismutase Levels (U/mL)			p-value
	Before Intervention Average + Deviation	After Intervention Average + Deviation	Average Difference	
Obesity Groups	2,33 $\pm$ 1,26	3,45 $\pm$ 0,54	1,12	0,652
Normal Groups	2,61 $\pm$ 1,11	3,76 $\pm$ 0,64	1,15	

Note: Numbers with different letters in the same column indicate significant differences between the subjects ($p < 0,05$).

Based on the table, it can be seen that the average value of serum MDA levels before intervention in the obesity group is lower than the normal group, but the average value of serum MDA levels after intervention in the obesity group is higher than the normal group. It can be concluded that, although there was a decrease in each group, there was a significant difference between the normal and obese groups.

The results of statistical tests showed that the decrease in serum MDA levels after intervention in the obese group ($-242,6 \pm 205,33$

ng/mL) was significantly ($p < 0,05$) higher than the normal group. This shows that the consumption of functional cookies has a significant impact on students who are overweight/obese because of the mechanism of reducing MDA caused by the antioxidant content of functional cookies. The antioxidant content of cookies also plays an important role in counteracting free radicals by breaking the chain of free radical oxidation reactions.

Antioxidants are compounds that inhibit oxidation at low levels (Nisa, 2017). In cookie products, antioxidant content can prevent

oxidative damage to target molecules. Antioxidants neutralize and release free radicals (Cherubini et al., 2005). Antioxidant status can represent the inhibition of free radicals by antioxidants in sample tissues such as serum or plasma. This antioxidant defense system includes enzymes and non-enzymatic antioxidants. The mean value of serum enzyme antioxidant activity, in this case, SOD, based on subject groups, is presented in Table 3. Based on the table, it can be seen that the mean value of serum SOD levels before intervention in the obese group was lower than that in the normal group, as well as the mean results of serum SOD after intervention. Based on the work of Ozata et al. (2002), SOD activity in obese individuals was significantly lower than that in those with normal body weight. It can be concluded that although there was an increase, there was no significant difference ($p>0,05$) between the normal and obese groups.

Before the intervention, it was found that the average value of antioxidant activity of the SOD enzyme in the serum of obese subjects was lower ($2,33 \pm 1,26$ U/mL) compared to the normal group, namely ($2,61 \pm 1,11$ U/mL). Low SOD activity indicates oxidative stress in the body, where antioxidant enzymes are unable to eliminate many oxidants (free radicals). Beltowski et al. (2000), found that in animal models that were obese, SOD enzyme activity was 29-42% lower than controls. In this study, the SOD activity in the obese group was lower than that in the normal group. If obesity persists for a long time, there will be a decrease in antioxidant sources such as superoxide dismutase (SOD) enzyme activity (Rahmawati, 2014).

The body has the ability to produce antioxidants naturally known as endogenous antioxidants, such as superoxide dismutase (SOD) (Irawan, 2013). Superoxide dismutase (SOD) is a bioactive substance that acts as an antioxidant and protects cells from oxidant (free radical) disorders that can cause various diseases (Yanti et al., 2016). SOD activity increases following the induction of enzymes by chemicals (Groppe et al. 2009).

The mean SOD activity in the obese and normal groups after the intervention was higher than before the intervention. Statistical tests showed that the results of SOD activity for the obese group and the normal group both showed an increase in SOD activity levels, although the

difference was not significant ($p>0,05$). This shows a tendency for the effect of providing functional cookies on SOD activity in obese students. The mechanism of increasing SOD enzyme activity occurs because antioxidants in functional cookies protect cells from inflammation caused by free radicals. SOD formation becomes more effective, and its activity increases by consuming mineral nutrients such as copper (Cu), manganese (Mn), and zinc (Zn) (Nisa, 2017). Insignificant statistical tests are likely due to the insufficient length of time given cookies to the subjects; therefore, it is necessary to increase the study time to give the product.

Systemic oxidative stress in the bloodstream increases due to increased ROS production from adipose tissue, which can damage other organs, such as the aorta, liver, and skeletal muscle (Intantri, 2008). Therefore, it is necessary to modify the diet rich in antioxidants (Vincent and Taylor 2006; Savini et al. 2013; Montero et al. 2011). Functional cookies to research subjects have been shown to improve the oxidative stress status of obese subjects, especially in significantly reducing MDA levels, while increasing SOD activity before and after the intervention, although not yet significant.

This study has limitations, namely that it did not involve a control group that was not given cookies or a group that was given cookies that did not contain antioxidants. This study used a fat group consisting of overweight and obese individuals due to the limited number of subjects who were obese.

Conclusion

Providing functional cookie products significantly reduces serum malondialdehyde (MDA) levels in obese and healthy adolescents. Superoxide dismutase (SOD) enzyme activity increased after the administration of functional cookie products to overweight/obese and normal adolescents. Further studies are needed to elucidate the mechanism underlying increased SOD activity in vivo.

The formulation of functional cookies from local food ingredients as a snack product needs to be socialized because it can contribute to controlling oxidative stress in teenagers who are overweight/obese to

prevent the emergence of various degenerative diseases.

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