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Research Article

The Potential of Cream Soup Made from a Combination of Black Soybeans (*Glycine soja L. merrit*) and Red Rice (*Oryza nivara*) to Improve Lipid Profiles and Control Oxidative Stress among Menopausal Women

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Abstract

Background and Objective: The number of menopausal women is estimated to continue to increase; therefore, as more women experience menopause, more health problems will emerge as a consequence. Black soybeans and red rice are known to have a high antioxidant content that can reduce the effect of free radical damage and prevent degenerative diseases in menopausal women. The objective of this study was to examine the potential of a cream soup made from a combination of black soybeans and red rice to improve lipid profiles [cholesterol total, triglycerides, High Density Lipoprotein-Cholesterol (HDL-C) and Low Density Lipoprotein-Cholesterol (LDL-C)] and control oxidative stress markers [malondialdehyde (MDA) and superoxide dismutase (SOD)] among menopausal women. **Materials and Methods:** This was an experimental trial with a crossover design. Eight menopausal women in Ciherang village, Dramaga Subdistrict in Bogor, West Java, were randomly assigned to the control group (n = 4) and the treatment group (n = 4) for 2×4-week periods of daily treatment with a 4-week washout period between the intervention periods. The treatment group consumed approximately 50 g of cream soup made from black soybeans and red rice, while the control group consumed cream soup made from white rice. Blood samples were taken in the morning on days 1, 29, 43 and 71 of the intervention and analyzed for serum cholesterol, triglycerides, HDL-C, LDL-C, MDA and SOD levels. **Results:** The results showed that the differences in cholesterol and triglyceride levels between the post-and pre-intervention periods in the treatment group were not significantly different from those in the control group. The differences in HDL-C and LDL-C levels between the post- and pre-intervention periods in the treatment group were significantly different from those in the control group. The difference in serum MDA levels between the post- and pre-intervention periods, which had decreased in the treatment group, was significantly different from that in the control group, which had increased ($p<0.05$). The difference in serum SOD levels between the post-and pre-intervention periods, which had increased in the treatment group, was significantly different from that of the control group, which had decreased ($p<0.05$). **Conclusion:** This study revealed that a functional cream soup made from a combination of black soybeans and red rice has the potential to improve lipid profiles and control oxidative stress among menopausal women.

Key words: Black soybeans, cream soup, lipid profile, menopausal women, oxidative stress, red rice

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The number of menopausal women is expected to continue to increase. A total of 477 million women worldwide in 1998 had experienced menopause. This number is estimated to increase to 1.1 billion by 2025; therefore, more women will be menopausal, with the consequence of having more health problems[1]. Menopause is a natural physiological process in women that occurs from the ages of 45-55 years. It is characterized by permanent cessation of menstruation due to ovarian failure to produce ovum and related hormones[2]. Menopausal women have various cardiometabolic risk factors, such as changes in body fat distribution, reduced glucose tolerance, abnormal plasma lipids, elevated blood pressure and the effects of decreased estrogen levels[3]. The results of the previous studies have shown that there is an increase in the prevalence of dyslipidemia among menopausal women. The waist-hip ratio and the levels of triglycerides, total cholesterol and LDL-C are higher in menopausal women than in premenopausal women. There is also a decrease in HDL-C in menopausal women[4,5]. In addition, menopausal women are at a 1.36-times greater risk [95% Confidence Interval (CI), Odds Ratio (OR) = 1.15-1.60] of having degenerative diseases (especially cardiovascular disease) than premenopausal women. The risk will increase 2-3 years after menopause[5,6].

Oxidative stress occurs due to the release of reactive oxygen species (ROS) in various tissues. It develops into various menopausal symptoms and pathological conditions, particularly cardiovascular disease[7]. Free radical damage or lipid peroxidation can be detected by the presence of MDA an indicator of lipid peroxidation in the blood[8]. Oxidative stress is indicated by low antioxidant status and decreased SOD levels, which are markers for primary antioxidants[9]. Several studies have confirmed that the consumption of high-antioxidant food products may inhibit lipid peroxidation and increase antioxidant enzymes[10].

Black soybeans are one of the high-antioxidant foods. The main functional components in black soybeans are isoflavones and anthocyanins. Isoflavones exhibit an estrogen-like role; therefore, they are often referred to as phytoestrogens[11]. Anthocyanin is one of the antioxidant components in black soybeans that can capture free radicals, inhibit lipid peroxidation and inhibit DNA damage[12]. The antioxidants in red rice derived from anthocyanin pigments are known to have the ability to prevent coronary heart disease, cancer, diabetes and hypertension[13-15].

Thus, a cream soup has been developed as an alternative functional food for menopausal women; it is made from a combination of black soybeans and red rice containing nutrients required by the body and substances that have potential as antioxidants. This product was served in this intervention study to assess its potential for improve lipid profiles and control the oxidative stress in menopausal women. The cream soup intervention was expected to decrease the total cholesterol, triglycerides and LDL-C levels as well as to increase HDL-C levels, decrease MDA levels and increase the SOD activity in menopausal women.

This study aimed to examine the potential of a cream soup made from a combination of black soybeans and red rice to improve lipid profiles and control oxidative stress in menopausal women.

MATERIALS AND METHODS

Design, time and location of the study: This study was an experimental trial with a crossover design. It was part of a dissertation research study entitled "The Potential of Cream Soup in Improve Lipid Profiles and Controlling Oxidative Stress among Menopausal Women". The study was conducted from March to December 2017. The research subjects received two treatments, namely, the cream soup intervention and a control. The study implementation was divided into three periods as follows: (1) the first phase of intervention period, (2) a washout period and (3) the second phase of the intervention period. Each phase of intervention was conducted for 28 days with a 2-week washout period. The subjects in the treatment group received cream soup made from a combination of black soybeans and red rice, while the control subjects received cream soup made from white rice flour. The cream soup was made at the Southeast Asia Food and Agricultural Science and Technology (SEAFAST) Center and Food Testing Laboratory, Nutrition Science Major, Bogor Agricultural University. The research briefing of and blood collection from the subjects were conducted in Posbindu in Ciherang Village, a working area of Puskesmas Dramaga, Dramaga Subdistrict, Bogor Regency, which was located in West Java, Indonesia. Serum lipid profile analysis was performed at the Regional Health Laboratory (LABKESDA) in Bogor City, while MDA levels and SOD analyses were performed at the Physiology Laboratory, Faculty of Medicine, Brawijaya University, which is located in Malang, East Java, Indonesia. This study has received ethics approval from the Health Research Ethics Committee of the Faculty of Medicine, University of Indonesia, Number: 435 /UN2.F1/ETIK/2017 dated May 8, 2017. The study subjects were served 50 g of cream soup flour for two daily snacks. The cream soup was prepared with 100 mL of warm water for one

brewing and the unconsumed cream soup was returned to the research team. During the intervention, the subjects receiving medications continued to take the drugs given by their physician.

Study materials and instruments: The materials used in this study were the cream soup prepared by the researchers based on the results of a study on the development of a cream soup made from a combination of black soybeans and red rice as a functional food for menopausal women. This product was made from black soybeans, red rice, chicken broth, carrots, skim milk, margarine, onion, garlic, spring onion, bay leaves, pepper, salt and sugar. The product had a slightly brownish color, the aroma of the black soybean and red rice mixture, a distinctive broth aroma, a slightly savory and salty taste and a soft texture in the mouth.

One serving (50 g) of the cream soup made from black soybeans and red rice contained approximately 202.46 kcal of energy that was estimated from the following equation: [(4×protein content)+(9×fat content)+(4×carbohydrate content)]. The protein content of the cream soup was 13.36 g. The daily energy and protein requirements at the age of menopause are 1900 kcal and 57 g, respectively. Thus, the cream soup product can contribute 10.66 and 11.71% of the recommended dietary allowances (RDA) for energy and protein for menopausal women[16]. The cream soup product made from black soybeans and red rice contained 17.87 g of total dietary fiber. The cream soup is considered a high-in-fiber food because it meets the requirement of the Ministry of Health stating that a food product can claim to be "High in fiber" if it contains 6 g of fiber/100 g in solid form[17]. Sufficient intake of dietary fiber is good for women of menopausal age. This cream soup product also contained 162.59 μg g^{-1} of total isoflavones and 254.58 μg g^{-1} of total anthocyanins. The antioxidant activity of the cream soup product was 723.73 mg 100^{-1} g, which was equivalent to that of vitamin C. These results indicated that 100 g of the cream soup product could reduce the DPPH free radical, which was equivalent to 723.73 mg 100^{-1} g of vitamin C. The total microbes in the cream soup product were 3.4×10^2 colonies/g. The result was still below the cut-off point (1×10^4 colonies/g) for such processed products; therefore, it was safe for consumption[18]. In addition, the control cream soup was prepared. It was formulated to match the intervention cream soup regarding form, taste and texture as closely as possible. However, the control cream soup had different total isoflavones, total anthocyanins and total dietary fiber contents.

The instruments used during the intervention were a microtoise, a bioelectrical impedance analysis (BIA) system, a sphygmomanometer, equipment for distributing the cream soup during the intervention and equipment for blood collection, lipid profile analysis and analysis of oxidative stress markers of the subjects.

Research subjects: The study subjects were menopausal women in Ciherang Village, a working area of Puskesmas Dramaga, Bogor, West Java, Indonesia. The inclusion criteria in this study were as follows: 1) menopausal women with a menopause period between one to five years; 2) a naturally occurring menopause period; 3) one abnormal blood lipid profile finding (total cholesterol >200 mg dL^{-1}, LDL-C >130 mg dL^{-1}, triglycerides >150 mg dL^{-1}, or HDL-C <40 mg dL^{-1}) or having hypertension (systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mm Hg); 4) age between 50 and 60 years; 5) have known or have consumed cream soup and 6) willing to be a respondent, to comply with the rules made during the study and to sign the informed consent form. The total subjects in this study numbered eight women.

Data types and data collection methods: The data collected in this study were the subjects' characteristics, including age, body weight, body height, physical activity, family history, drug type and drug consumption, which were obtained from an interview. During the cream soup intervention, data regarding the subjects who consumed the cream soup were collected. The lipid profile data included the total cholesterol, triglycerides, LDL-C and HDL-C levels. We collected data on oxidative stress markers, which included the MDA and serum SOD levels of the subjects.

Data regarding the characteristics of subjects were collected before the intervention. The age and physical activity data were collected through the interview using questionnaires as an aid. Body weight was measured with a step-on digital scale with 0.1 kg accuracy and 200 kg capacity. Body mass index (BMI) was assessed through body weight and height indicators. Blood pressure was measured on the right hand after the subjects sat for 15 min. The measurements were performed three times at 5 min intervals and the mean was calculated from the three blood pressure data points obtained. Body height was measured using a microtoise with an accuracy of 0.1 cm and a length of 200 cm.

Data on the subjects' compliance in consuming the cream soup were collected through observation and interview, using an aid, namely, a monitoring form of product consumption

compliance. Blood was collected from the subjects to determine the lipid profile (total cholesterol, triglycerides, LDL-C and HDL-C levels), MDA levels and serum SOD levels.

Blood collection was performed four times, on days 1, 29, 43 and 71. The subjects were asked to fast at least 10 h, starting the night before until the next morning prior to the blood being taken. The 10 mL venous blood sample was collected from the median cubital vein (antecubital fossa) using a vacutainer containing EDTA anticoagulant. The blood collection was conducted at Puskesmas Darmaga by health analysts who had certificates of competence. A 10 mL blood sample was taken using a disposable syringe. The sample was then immediately put in a sterile Falcon tube and centrifuged at 3000 rpm for 10 min. Centrifugation was conducted at the Biochemistry Laboratory of Nutrition Science Major, Bogor Agricultural University. The next study phase (blood biochemical analysis) was performed on the serum obtained through fractionation of the subjects' blood samples, which were collected by the health analysts before and after the intervention. The blood samples were immediately brought to the laboratory for serum lipid profile analyses, which included the analysis of total cholesterol levels (CHOD-PAP enzymatic colorimetric method with a spectrophotometer at 500 nm wavelength), triglycerides levels (GPO-PAP colorimetric method with a spectrophotometer at 500 nm wavelength) and HDL-C levels (precipitation method with a spectrophotometer at 546 nm wavelength). The LDL-C levels were then calculated with the following formula: [total cholesterol – triglycerides – HDL-C and LDL-C]. The serum lipid profile analysis was performed at LABKESDA in Bogor City, West Java. The serum MDA levels were analyzed with the thiobarbituric acid reactive substances (TBARS) assay using a spectrophotometer at 532 nm wavelength. The SOD activity was analyzed using a spectrophotometer at 480 nm wavelength at the Physiology Laboratory of Faculty of Medicine in Brawijaya University, Malang. Secondary data (diagnosis of dyslipidemia) were obtained from the Puskesmas and local Posbindu cadres.

Statistical analysis: The data were analyzed using computer software. The descriptive statistics included mean, median, standard deviation and minimum and maximum values were determined. The significance level used in this study was 0.05. The paired t-test was performed to examine the difference between the control and treatment groups before and after the intervention. An independent t-test was performed to determine the potential of the cream soup made from a combination of black soybeans and red rice to improve lipid profiles and control oxidative stress.

RESULTS

Subjects' characteristics: A total of eight subjects participated in all study phases. The characteristics of subjects can be seen in Table 1. The mean age of the subjects was 57±4.63 years with an age range of 50-60 years. The mean BMI of the subjects was 27.55±2.11 kg m^{-2}, which was categorized as overweight. Based on PAL, the physical activity of the subjects was categorized as light with a mean value of 1.53±0.10. Based on the mean systolic (147.5±24.29 mmHg) and diastolic blood pressure (86.5±10.67 mmHg), the subjects were categorized as having hypertension.

Compliance with cream soup product consumption: The compliance with cream soup consumption was assessed from the amount of cream soup consumed in a day. The subjects had good compliance because the mean amount of cream soup consumed each day was greater than 95%. The mean amounts of the intervention and the control cream soup consumed for 28 days in the first phase and for 28 days in the second phase were 96.76±4.45 and 95.98±6.45%, respectively. The amount of cream soup consumed by the treatment group was higher than that by the control group but there was no significant difference between the two groups ($p>0.05$). The reasons why the subjects did not consume the intervention product were because they forgot or they were traveling out of town.

Potential of cream soup made from a combination of black soybeans and red rice to improve lipid profiles: Based on the study results presented in Table 2, the mean cholesterol levels in the treatment group decreased significantly by 44.1±51.3 mg dL^{-1} after the intervention, while the mean cholesterol level in the control group increased by 0.87±32.3 mg dL^{-1}. However, there was no significant difference in the mean difference of the cholesterol levels between the control and the treatment groups ($p>0.05$). The mean triglyceride levels decreased by -14.6±51.3 mg dL^{-1} in the treatment group and increased by 9.0±28.6 mg dL^{-1} in the control group after the intervention. The mean difference of triglyceride levels between the control and the treatment groups was not significantly different ($p>0.05$).

Table 1: Subjects' characteristics

Variables	Value (Mean±SD)	Range
Age (years)	57±4.63	50-60
BMI (kg m^{-2})	27.55±2.11	25.1-29.9
Physical activity (PAL)	1.53±0.10	
Blood pressure (mmHg)		
Systolic	147.5±24.29	110-130
Diastolic	86.5±10.67	70-90

Table 2: Mean serum lipid profile of the subjects in the treatment group and control group

		Serum lipid profile of the subjects			
Variables	Normal level	Treatment	Control	p^1	p^2
Cholesterol (mg dL^{-1})	<200				
Before intervention		230.5±37.7	214.6±21.3	0.054	0.045*
After intervention		186.4±22.4	215.5±35.9		0.941
Difference		-44.1±51.3	0.87±32.2		
Triglycerides (mg dL^{-1})	<150				
Before intervention		134.5±41.1	119.8±26.1	0.274	0.447
After intervention		119.8±43.6	128.8±23.7		0.403
Difference		-14.6±51.3	9.0±28.6		
HDL-C (mg dL^{-1})	>40				
Before intervention		54.0±14.6	66.9±7.8	0.022*	0.677
After intervention		55.5±13.3	55±13.5		0.018*
Difference		1.5±9.7	-11.8±10.9		
LDL-C (mg dL^{-1})	<100				
Before intervention		149.5±34.6	123.8±18.6	0.016*	0.043*
After intervention		107±25.3	134.8±30.2		0.269
Difference		-42.5±48.7	11.0±25.9		

[1]Independent t-test, [2]paired t-test), *Significant with p<0.05

Table 3: Mean serum MDA and SOD levels of the subjects in the treatment group and the control group

	Mean serum MDA and SOD levels of the subjects			
Variables	Treatment	Control	p^1	p^2
MDA (ng mL^{-1})				
Before intervention	196.75±33.06	321.76±20.23	0.000*	0.050*
After intervention	155.50±36.64	412.16±16.32		0.000*
Difference	-41.25±49.31	90.4±24.3		
SOD (U mL^{-1})				
Before intervention	25.08±1.59	2.46±3.93	0.015*	0.120
After intervention	27.55±4.19	23.22±2.15		0.065
Difference	25.96±2.14	-2.73±3.54		

[1]Independent t-test, [2]paired t-test), *Significant with p<0.05

The mean HDL-C levels increased by 1.5±9.7 mg dL^{-1} after the intervention in the treatment group but the increase was not significant (p>0.05). Meanwhile, the mean HDL-C levels decreased significantly by -11.8±10.9 mg dL^{-1} in the control group. There was a significant difference in the mean difference of HDL-C levels between the control and the treatment groups. The mean LDL-C levels decreased significantly by -42.5±48.7 mg dL^{-1} after the intervention in the treatment group and increased by 11.0±25.9 mg dL^{-1} in the control group. The mean difference of LDL-C levels between the control and the treatment groups was significantly different (p<0.05).

Potential of cream soup made from a combination of black soybeans and red rice to control oxidative stress: In Table 3, it can be seen that the mean values of the serum MDA levels of the treatment group before and after the intervention were lower than those of the control subjects. The mean value of the serum MDA levels after the intervention in the treatment group was significantly lower (p<0.05) than that before the intervention. The mean serum MDA levels in the control subjects were significantly different (p<0.05) between the pre- and post-intervention periods. The results of the statistical analysis indicated that the decrease in the serum MDA levels in the treatment group (-41.25±49.32 ng mL^{-1}) was significantly higher (p<0.05) than the decrease in the control subjects (90.4±24.3 ng mL^{-1}).

Before the intervention, the mean value of the antioxidant enzyme activity of SOD in the serum of the treatment group (25.08±1.59 U mL^{-1}) was lower than that of the control subjects (25.96±2.14 U mL^{-1}) (Table 3). The low SOD activity proved that the oxidative stress condition in the body occurred because the antioxidant enzymes were not able to eliminate the number of oxidants. After the intervention, the mean value of the antioxidant enzyme activity of SOD in the serum of the treatment group (27.55±4.19 U mL^{-1}) was higher than that of the control subjects (23.22±2.15 U mL^{-1}). The statistical analysis showed that the mean difference of the antioxidant enzyme activity of SOD in the treatment group (2.46±3.93 U mL^{-1}) was significantly higher (p<0.05) than that in the control subjects (-2.73±3.54 U mL^{-1}).

DISCUSSION

Dyslipidemia is an abnormal amount of lipid and lipoprotein in the blood characterized by elevated levels of total cholesterol, triglycerides and LDL-C and low levels of HDL-C. It is one of the causal factors of atherosclerosis, thereby increasing the risk of cardiovascular disease[19].

Table 2 shows the mean difference of total cholesterol, triglycerides and LDL-C levels in the treatment group were lower than that of in the control group, while the mean HDL-C level was higher in the treatment group than in the control group. There was a decrease in the total cholesterol, triglycerides and LDL-C levels after the intervention of cream soup made from a combination of black soybeans and red rice in the subjects receiving the intervention. Some reports suggested that the antioxidant intake might affect the mechanism in the pathogenesis of dyslipidemia. Various forms of antioxidants (i.e., vitamin E, ascorbic acid and isoflavones) are known to prevent the oxidation of cholesterol, triglycerides and LDL-c[20]. The results of this study were consistent with a study on an isoflavones intervention that significantly decreased the serum total cholesterol levels by 0.10 mmol L^{-1} (3.9 mg dL^{-1} or 1.77%; $p = 0.02$) and LDL-C levels by 0.13 mmol L^{-1} (5.0 mg dL^{-1} or 3.58%; $p<0.0001$); in addition, the isoflavones also significantly increased the HDL-C levels by 0.04 mmol L^{-1} (1.6 mg dL^{-1} or 3.00%; $p = 0.05$)[21].

Another study suggested that an antioxidant intervention could significantly decrease the total serum cholesterol level by 3.77%, LDL-C by 5.25% and triacylglycerol by 7.27% and significantly increase the HDL-C levels by 3.03%[22]. In addition, antioxidants can lower the plasma cholesterol levels by inhibiting cholesterol absorption in the gut and increasing the bile acid formation from cholesterol, which is then excreted through the feces[23].

Antioxidants can influence the enzyme activity of lipoprotein lipase (LPL) to hydrolyze triglycerides in chylomicrons and convert them into free fatty acids, which are then stored in adipose tissue[24]. In addition, the phytochemical substances (cyanidin-3-glucoside) in black soybeans can reduce the total cholesterol and LDL-C levels and increase HDL-C levels by increasing LDL-C receptors[12,25].

Oxidative stress is oxidative damage caused by the continuous accumulation of dangerous molecules, resulting in an imbalance between pro-oxidants and antioxidants that leads to lipid or protein peroxidation. The lipid peroxidation produces various end products that are relatively stable and most of them are aldehydes such as MDA[26]. Oxidative stress is indicated by the low antioxidant status and level of SOD, which is the marker for antioxidants. The body's efforts to counteract the oxidation reaction are carried out by endogenous antioxidants (e.g., vitamins and enzymes) and exogenous antioxidants (from food).

Table 3 shows the administration of cream soup made from a combination of black soybeans and red rice had a significant effect on the decrease in serum MDA levels among menopausal women receiving the intervention. This condition is in line with the results of a study on the ability of antioxidants to decrease MDA levels in the treatment group[27]. MDA is the main product of the reaction between free radicals and phospholipids. The antioxidants contained in the functional cream soup made from a combination of black soybeans and red rice are substances that can inhibit oxidative damage to the target molecules. The antioxidant molecules can react with free radicals[28]. A review of several studies regarding menopausal women and oxidative stress, including one study that involved 102 menopausal respondents, showed higher MDA levels in obese women than in subjects who were underweight[29]. The administration of isoflavones through soy milk was able to decrease the MDA levels significantly ($p = 0.02$). The results of this study were consistent with that of the previous study, indicating that a high-antioxidant diet could decrease MDA levels[30]. The decrease in the MDA levels might occur due to the antioxidant content (isoflavones) in the cream soup product that played a role in warding off free radicals by cutting the chain reaction of free radical oxidation[31]. Antioxidants that act as free radical scavengers have hydroxyl (OH-) groups in aromatic rings. They stop the chain reaction of lipid peroxidation by protecting cells and chemical elements in the body[32].

Antioxidants are molecules that can prevent the negative effects of the oxidation process and keep the cells and tissues free from radical damage[30]. Isoflavones in soybeans that act as primary antioxidants can directly eliminate free radicals and increase the concentration of antioxidant enzymes. In addition to the isoflavones, anthocyanins in the cream soup have chemical structures that can be reactive to ROS due to electron deficiency and have the potential to sustain the SOD levels in the blood by modulating the hepatic SOD mRNA[33,34].

SIGNIFICANCE STATEMENT

This study found that the cream soup made from black soybeans and red rice can benefit menopausal women. The study showed that antioxidants contained in black soybeans (isoflavones) and red rice (anthocyanins) can improve and control oxidative stress in menopausal women. Therefore, this product can serve as an alternative functional food or health food for menopausal women.

CONCLUSION

The administration of cream soup made from a combination of black soybeans and red rice has the potential to significantly improve lipid profiles by increasing the HDL-C levels and decreasing the LDL-C levels. However, the cream soup did not significantly decrease the total cholesterol and triglycerides levels in menopausal women compared to control subjects. In addition, administration of cream soup made from a combination of black soybeans and red rice has the potential to significantly control oxidative stress by decreasing the serum MDA levels and increasing the SOD enzyme activity significantly in menopausal women compared to administration of the cream soup made from white rice in control subjects.

RECOMMENDATION

Menopausal women can consume as a snack a cream soup made from a combination of black soybeans and red rice, which is beneficial for health, with a daily dose of 50 g of the flour. The cream soup can increase the HDL-C and SOD levels and decrease the LDL-C and MDA levels.

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