ABSTRACT

Red beans are legumes with the highest carbohydrate content potentially as a source of resistant starch (RS). RS is proven to be beneficial for various physiological functions of human body, specifically its hypocholesterolemic properties which can reduce blood cholesterol. The use of red beans is still limited because of the long time processing. These problem can be overcome by processing as pregelatinization red bean flour. The resulting flour is high in RS, longer shelf life, easy to mix, easy to shape and more applicable. Pregelatinization with the combined heating-cooling method is one of the efforts to increase the level of RS which is environmentally friendly. Several studies of pregelatinized red bean flour have been carried out but RS levels have not been optimal. Most red bean research is still starch-based, although legume starch is rarely consumed on its own. Therefore, this study aimed to obtain RS high pregelatinized red bean flour through modified heating-cooling combine process that had a hypocholesterolemic effect and improved composition of the colonic bacteria.

Red beans were obtained from traditional markets in Magelang, Central Java, Indonesia. The research consisted of 3 stages, namely: 1) processing of pregelatinization red bean flour with a combination heating-cooling method, with the treatment: (i) steaming-cooling 3 cycles (SSS), (ii) steaming-cooling, oven microwave-cooling, autoclaving-cooling (SMA) and (iii) steaming-cooling, autoclaving-cooling, oven microwave-cooling (SAM). Steaming for 30 minutes boiling, microwave 2450 Hz, 900 W for 4 minutes and autoclaving 121 °C, 15 atm, 145 seconds; 2) *in vivo* test (hypocholesterolemic properties, characteristics of cecum digesta, SCFA composition and colonic microbiota composition)and 3) *in vitro* test (bile acid binding capacity) RS high red bean flour. *In vitro* and *in vivo* tests used two types of diets, which consisted of a natural red bean flour (HTN) diet and a modified red bean flour (HTM) diet. In the *in vivo* test, isocalories & isoprotein feeds were prepared for 2 groups of SD rats, namely the group given the HTN & HTM diet. Healthy rats on a standard diet AIN 93 (NPS), hypercholesterolemic rats on a standard diet (HPS) and hypercholesterolemic rats on a standard diet with statin treatment (HPV).

The results showed that red bean flour pregelatinized SMA produced the highest RS content compared to SSS and SAM. Successively increasing levels of RS 116.10%db; 89.62%db and 86.05%db. This modified treatment resulted in decreased swelling, WHC, OHC, solubility, and pasting properties and changed the microscopic structure. Scanning electron microscopy showed flakes of starch granules leading to loss of granular appearance, rough surface, irregular shape, larger size and heterogeneity. X-ray diffraction analysis showed that all red bean flour had type C crystallinity. In vitro testing showed that the binding capacity of bile acids was more significant the HTM diet than the HTN diet, with an increase in the binding of cholic acid 13.61% and deoxy cholic acid 48.02%. *In vivo* testing of the HTM group, it could improve the lipid profile of dyslipidemic rats, with a decrease in TC, LDL, TG, respectively 43.66%; 57.8% and 16.92% and could increase HDL by 171.61%. Meanwhile, IA decreased to 78.65%. HTM diet could increase the total SCFA by 152.43% and propionic acid 155.29%. The HTM diet could increase the composition of *Bifidobacterium, spp* and *Lactobacillus, spp* and reduce the number of *E. coli* bacteria. The mechanism for improving the lipid profile of HTM diet in dslipidemic rats was proven by: (1) inhibition of cholesterol absorption, (2) increased excretion of bile acids, (3) increased propionic acid in digesta,

or a combination of the three mechanisms. The improvement of colonic bacteria composition through the physical adhesion mechanism of Bifidobacterium to the RS and its fermentation produces energy modulating the growth of probiotic bacteria, acidic conditions suppress the growth of pathogenic bacteria.

Pregelatinized red bean flour (SMA) produced the highest RS compared to other modifications and natural red bean flour. The HTM diet provided a better lipid profile repair effect than the HTN diet and could improve the colonic microbiota composition of dyslipidemic rats.

Keywords: red bean flour, pregelatinization, heating-cooling combination, hypocholesterolemic, lipid profile, colonic bacteria.