Mal J Nutr 23(3): 461 - 471, 2017

# Antidiabetic and Immunomodulatory Potential of Purple Soymilk Enriched with Crude Palm Oil Microcapsule in Type-2 Diabetes Mellitus Respondents

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## ABSTRACT

**Introduction:** Purple soymilk produced from black soybean has been reported to reduce fasting blood glucose (FBG) and HbA1c of type 2 diabetes mellitus (T2DM) patients. Crude palm oil (CPO) is known to have immunomodulatory activity. This study aimed to investigate the antidiabetic and immunomodulatory potential of purple soymilk enriched with CPO microcapsule (PSPOM) in T2DM respondents. **Methods:** A total of 25 T2DM patients from Katili clinic and Bogor Agricultural University, Indonesia were recruited by purposive cluster sampling, and divided into control and test groups. The test group consumed PSPOM, made up of 240 mL of purple soymilk with 0.4 g CPO microcapsule, containing 295.24 $\mu$g carotenoid/g capsule daily for 4 weeks. Blood sample of 2 mL was collected from each subject before and after the intervention. Dietary intakes were assessed using the 24-h food recall method. **Results:** When compared between the start and end of the intervention, the test group showed significant mean decreases in fasting blood glucose from 177.20 ± 93.55 to 154.87 ± 71.13 mg/dL, and in HbA1c from 0.29 ± 0.07 to 0.26 ± 0.06 $OD_{450}$. The test group also showed a mean increase of IL-6 level from 0.23 ± 0.04 to 0.25 ± 0.06 $OD_{450}$, and significant increases in the level of insulin from 0.18 ± 0.05 to 0.21 ± 0.07 $OD_{450}$ over the same duration. Results also showed increases of $CD4^+$ from 563.13 ± 194.62 to 679.95 ± 278.86 cell/$\mu$L blood, and $CD8^+$ T cells from 317.91 ± 105.40 to 343.74 ± 162.27 86 cell/$\mu$L blood during the intervention period. **Conclusion:** Consumption of PSPOM in this study showed potential antidiabetic and immunomodulatory activities in T2DM respondents. Further studies should be undertaken to confirm these results.

**Key words:** Antidiabetic, crude palm oil, immune-modulatory activity, microcapsules, purple soymilk

## INTRODUCTION

Type 2 diabetes mellitus (T2DM) is characterised by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion and insulin action, or both. The global prevalence of T2DM has risen from 108 million in 1980 to 422 million

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in 2014 and more than 80% of T2DM deaths occur in low- and middle-income countries (WHO, 2016).

The metabolic characteristics of T2DM patients are abnormally high concentrations of glucose in the blood, a higher haemoglobin-A1c (HbA1c) level but abnormally low insulin levels (Sedaghat *et al*., 2015). Chronic hyperglycemia in the T2DM patient will stimulate the production of reactive oxygen species (ROS) and increase oxidative stress (Brownlee, 2005). The oxidative stress condition may increase levels of cytokines pro-inflammation such as interleukin (IL) and may stimulate cell death of various cell types including $CD4^+$ and $CD8^+$ T lymphocytes cells (Arya & Tripathi, 2011). Therefore, generally T2DM patients show an increase in IL-6 levels (Imai *et al*., 2013) and a reduction in $CD4^+$ T helper and $CD8^+$ cytotoxic lymphocyte levels (Abdelrazaq *et al*., 2013).

Black soybean (*Glycine soja* (L) *Merrit*) has been used as a traditional medicine in Asian countries for hundreds of years. Black soybean is rich in isoflavones, flavonoids, γ-tocopherol, and anthocyanins which possess biological activity (Jeng *et al*., 2010). Antioxidant properties of black soybean have been shown to be comparatively higher in black soybean than in the yellow soybean (Zakaria *et al*., 2016). Purple soymilk is a beverage made from black soybean and is rich in protein, fibre, oligosaccharides, isoflavones, anthocyanins, vitamins and minerals, with very low glycemic index (GI) (Zakaria, Firdaus & Yuliana, 2016). Soybean and their products have been demonstrated to reduce fasting blood glucose (FBG), HbA1c (Sedaghat *et al*. 2015), increase insulin-level in diabetics (Jayagopal *et al*., 2002), decrease IL-6 levels (Ji *et al*., 2012) and improve the immune system (Klein *et al*., 2002).

*β*-carotene is widely distributed in foods, especially in crude palm oil (CPO) and has a variety of different actions including protective role against the development of chronic diseases such as T2DM (Sluijs *et al*., 2015). There are various studies on the relationship between the intake of carotenoids with glucose metabolism and immunomodulators (Sluijis *et al*., 2015), and with an increase in number of $CD4^+$ lymphocytes (Kazi *et al*., 1997).

Benefits of yellow soymilk and palm oil to T2DM patients have been studied separately, however the intervention of purple soymilk enriched with CPO microcapsule in T2DM respondents have not been reported. Enrichment of purple soymilk lacking carotenoids with CPO microcapsules as the source of carotenoids would complement the bioactive compounds in purple soymilk. Thus, the aims of this study were to investigate the anti-diabetic and immunomodulatory activities of PSPOM in T2DM respondents.

## METHODS

### Preparation of purple soymilk enriched with CPO microcapsule (PSPOM)

Black soybean (*Glycine soja* (L) *Merrit*) from the Province of Lampung, Indonesia was washed with water repeatedly, soaked in water at a ratio of 1: 3 (w/v) for 12 hours and ground with the addition of water at 85 °C at the ratio of 1: 8 (w/v). The resulting soymilk, purple in colour was filtered with a 80 mesh filter cloth and pasteurised at 85°C for 15 min to obtain purple soymilk ready for consumption (Zakaria *et al*., 2016) and for further proximate analysis including moisture, ash, fat and protein contents (AOAC, 2005).

The CPO microcapsule was prepared by first mixing the coating mixture. Food grade maltodextrin (Zhucheng Dongxiao Biotechnology Co., China) and soy isolate protein (SPI) (MarkSoy 90, China) were mixed at a ratio of 2:1 (w/w), followed by addition of water at 60°C at a ratio of 1:3 (w/v), and by homogenising at 11.000 rpm for 1 min. The coating mixture was added with CPO from a local supplier (SMART Ltd, Indonesia) at a ratio of 1.0:1.6

(w/w), and homogenised at 12.000 rpm for 3 min followed by spray drying at inlet and outlet temperature of 180°C and 80°C, respectively. The CPO microcapsule formed was kept in a desiccator until further use.

Purple soymilk enriched with the CPO microcapsule (PSPOM) was prepared by adding 240 mL of purple soymilk with 0.4g CPO microcapsule, containing 295.24 µg carotenoid /g microcapsule (Hasrini *et al*., 2017). PSPOM was prepared freshly for each day of the intervention.

**Study design**

The study was carried out at Katili clinic and the Department of Food Science and Technology, Bogor Agricultural University, both located in Bogor, West Java, Indonesia. A total of 25 respondents were included in the study, of which two groups were formed: 10 (3 males and 7 females) T2DM respondents as the control group and 15 (6 males and 9 females) T2DM respondents who consumed the PSPOM as the test group. The respondents were selected based on inclusion criteria of fasting blood glucose (FBG) above 120 mg/dL and aged 20-75 years. Respondents diagnosed at the clinic with clinical signs of advanced complications, other chronic illnesses, allergy to soybean, smoking and pregnancy were excluded from the study.

PSPOM was given to the test group once a day during lunch time for 4 weeks while the control group did not receive PSPOM. During the study, respondents remained on their usual medications from the clinic. All the participants received counselling about healthy diets during each visit.to the clinic. Anthropometric, height and weight measurements were taken at the beginning and end of the intervention and were used for body mass index (BMI) calculation.

Every week during the intervention, nutrient intakes by both groups were assessed using the 24-h dietary food recall questionnaire. Macronutrients intake was calculated using the NutriSurvey software (EBISpro software) based on data from Indonesian food-composition tables (*http://www.nutrisurvey.de/databases.htm*). Blood withdrawal was done in the clinic by certified nurses at the beginning (baseline) and end of the 4 weeks of intervention. Fasting blood glucose (FBG) level was measured by prick method using glucometers. Blood samples of 2 mLs were collected after 12 h fasting in vacutainer tubes containing $K_2$EDTA as the anticoagulant. One mL of the blood sample was separated for the analysis of $CD4^+$ and $CD8^+$ T cells by flow cytometry method. The rest of the blood was centrifuged to analyse HbA1c, insulin, and IL-6 levels in the plasma by ELISA indirect method (Zakaria *et al*., 2014).

**HbA1C analysis**

For the HbA1c analysis (Zakaria *et al*., 2014), blood plasma was diluted with carbonate/bicarbonate buffer (Sigma Aldrich C3041) at a ratio of 1: 500, and then 100 µL was placed in a 96-well microplate (Nunc Maxisorp F96) and incubated at 4°C overnight. The liquid in the microplate was removed and washed for 1 min with 250 µL /well PBST solution (Sigma Aldrich P4417) containing 0.05% Tween 20 (Sigma Aldrich P1379). The washing process was done three times, then the wells of the plate were added with 100 µL 5% commercial skim milk powder solution and incubated at 37 °C for 1 h. The liquid in the microplate was then removed and washed with PBST three times before adding 100 µL of primary antibody in the wells (Mouse Anti-human HbA1c antibody, RES-ABIN791524) diluted with PBST at a ratio 1: 10.000 and then re-incubated at 37 °C for 1 h. The liquid in the microplate was removed and washed with PBST three times. Secondary antibody (HRP conjugated rabbit antimouse antibody, Genetex GTX26728) was diluted with PBST at a ratio of 1: 10.000 and then 100 µL was

added and incubated at 37°C for 1 h. After the liquid in the microplate was removed and washed, the wells were added with 50 µL of TMB substrate, then incubated at 37 °C for 15 min. After that, 2M $H_2SO_4$ of 50 µL was added as stop solution. The intensity of the colour formed was read with a microplate reader (Benchmark) at a wavelength of 450 nm.

**Insulin analysis**

Insulin levels were analysed (Zakaria *et al*., 2014) by the same procedure adopted for HbA1c with slight modifications. Plasma was diluted with carbonate/bicarbonate buffer (Sigma Aldrich C3041) 1: 1.000. The primary antibody used was mouse anti-human insulin antibody (Abcam RES-AB6995) that was diluted 12.000 times with PBST.

**IL-6 analysis**

IL-6 levels were also analysed (Zakaria *et al*., 2014) by the same procedure explained above. The primary antibody used for the analysis was mouse anti-human-IL-6 antibody, 10R-1787 (Fitzgerald RES-10R-1787) which was diluted at a ratio of 1: 12.000.

**$CD4^+$ and $CD8^+$ T cells analysis**

Each micro-tube was filled with 100 µL of whole blood sample and then added with 10 µL of R-phycoerythrin (PE) mouse anti-human CD3 reagent and 7.5 $\mu$L of fluorescein isothiocyanate (FITC) mouse antihuman CD4 (BD Biosciences PMG561842, USA) reagent; and then 5 $\mu$L of allophycocyanin (APC) mouse antihuman CD8 (BD Biosciences PMG561953, USA) (BD Biosciences, 2011). Each microtube was vortexed gently and incubated for 20 min at 25°C in a dark environment. Then, 450 $\mu$L of lysing solution was added, vortexed gently and incubated for 15 min at 25°C. The dilution was read by flow cytometer (BD ™ Accuri C6, USA). The data obtained was entered into the formula to obtain the level (absolute value) of $CD4^+$ and $CD8^+$ T cells per $\mu$L of blood. BD Accuri C6 software was used for data acquisition and analysis. The formula for counting absolute value of $CD4^+$ and $CD8^+$ T cells is given below:

$$CD4^+T\ cell\ absolute\left(\frac{cell}{\mu l\ blood}\right) = \frac{even\ CD4}{volume} \times dilution\ factor$$

$$CD8^+T\ cell\ absolute\left(\frac{cell}{\mu l\ blood}\right) = \frac{even\ CD8}{volume} \times dilution\ factor$$

**Statistical analysis**

All data were reported as mean ± standard deviation. The results were statistically analysed by the Statistical Package for Social Science (SPSS) 11.0 computer program. Differences between the control groups and the test groups were assessed using an independent *t*-test. P-values less than 0.05 were considered as statistically significant.

**Ethical statement**

The study was approved by the Institutional Ethical Committee of Research Institute and Public Service of Atma Jaya Catholic University Jakarta, Indonesia (Ethical Clearance No: 576/III/LPPM-PM.10.05/07/2014, on 2nd July 2014). The aims of the study were explained to all respondents who signed the informed consent documents.

## RESULTS

**Nutritional values of purple soymilk and CPO microcapsule**

The nutritional values of purple soymilk and CPO microcapsule (PSPOM) are shown in Table 1. This enriched PSPOM contained various nutrients including bioactive compounds except for digestible carbohydrate. The bioactive compound content of PSPOM in this study has been reported by other team researchers including total flavonoid (Meriyanti, 2015), free daidzein and genistein (Zuhri, 2015),

**Table 1.** Nutritional values of purple soymilk and CPO microcapsule in this study

| *Contents* | *Mean ± SD* |
|---|---|
| Purple soymilk | |
| Protein (%) | 2.76 ± 0.13 |
| Ash (%) | 0.12 ± 0.08 |
| Fat (%) | 1.17 ± 0.06 |
| Carbohydrate (%) | 1.27 ± 0.10 |
| Moisture (%) | 94.69 ± 0.04 |
| Total flavonoid (mg QE/100 g dw)[a] | 169.92±1.86 |
| Free daidzein ($\mu$g/g)[b] | 720.96±7.20 |
| Free genistein ($\mu$g/g)[b] | 27.05±0.00 |
| Total anthocyanin (mg C-3-G equivalents)[c] | 3.57±0.00 |
| CPO microcapsule: | |
| Total carotenoid ($\mu$g/g) [d] | 295.24±0.32 |

[a]:Meriyanti (2015); [b]: Zuhri (2015); [c]: Zakaria *et al.*(2016); [d]: Hasrini *et al.* (2017)

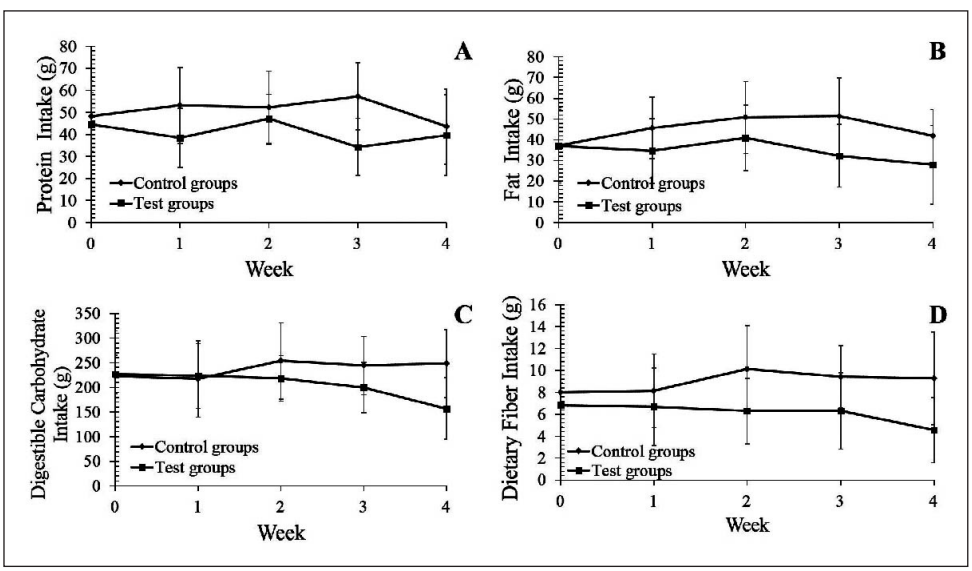


**Figure 1.** Protein (A), fat (B), digestible carbohydrate (C), and dietary fibre intake (D) of T2DM respondents. Control group was T2DM respondents without treatment; test group was T2DM respondents treated with PSPOM for 4 weeks.

anthocyanins (Zakaria *et al*., 2016) and carotenoid content of CPO microcapsule (Hasrini *et al*., 2017).

**Nutrient intake of the T2DM respondents**

Figure 1A until 1D show protein, fat, digestible carbohydrate and dietary fibre intake of control group and test group, respectively. At the beginning of intervention (week 0), nutrient intakes in both groups were similar. However, starting in the first week until the fourth week, test group intake of protein, fat, digestible carbohydrate and dietary fibre intake was lower than that of control group.

**Table 2.** The characteristics of the T2DM respondents and changes in metabolic profile from baseline until 4 weeks treatment

| | *Control groups* | *Test groups* | *P value* |
|---|---|---|---|
| Gender | | | |
| Male | 3 | 6 | NS |
| Female | 7 | 9 | NS |
| Age (year) | 55.00 ± 10.48 | 54.93 ± 6.92 | NS |
| Weight (kg) | 59.20 ± 6.50 | 56.90 ± 11.12 | NS |
| BMI (kg/m$^2$) | 26.18 ± 3.88 | 24.60 ± 3.84 | NS |
| FBG (mg/dL) | | | |
| Baseline | 256.70 ± 98.26 | 177.20 ± 93.55 | NS |
| 4 weeks | 212.20 ± 51.59 | 154.87 ± 71.13 | $p$<0.05 |
| Changes | -44.50 (17.33%) | -22.50 (12.60%) | $p$<0.05 |
| HbA1c ($OD_{450}$) | | | |
| Baseline | 0.42 ± 0.10 | 0.29 ± 0.07 | $p$<0.05 |
| 4 weeks | 0.42 ± 0.09 | 0.26 ± 0.06 | $p$<0.05 |
| Changes | 0.00 (0%) | -0.03 (10.34%) | $p$<0.05 |
| Insulin ($OD_{450}$) | | | |
| Baseline | 0.23 ± 0.04 | 0.18 ± 0.05 | $p$<0.05 |
| 4 weeks | 0.24 ± 0.03 | 0.21 ± 0.07 | $p$<0.05 |
| Changes | 0.01 (4.16%) | 0.02 (14.28%) | $p$<0.05 |
| IL-6 ($OD_{450}$) | | | |
| Baseline | 0.16 ± 0.03 | 0.23 ± 0.04 | $p$<0.05 |
| 4 weeks | 0.21 ± 0.04 | 0.25 ± 0.06 | NS |
| Changes | 0.05 (23.81%) | 0.02 (8.00%) | $p$<0.05 |
| $CD4^+$ T cells (cell/$\mu$L blood) | | | |
| Baseline | 428.25 ± 290.31 | 563.13 ± 194.62 | NS |
| 4 weeks | 411.58 ± 291.93 | 679.95 ± 278.86 | $p$<0.05 |
| Changes | -16.67 (3.89%) | 116.82 (17.18%) | $p$<0.05 |
| $CD8^+$ T cells (cell/$\mu$L blood) | | | |
| Baseline | 313.79 ± 165.69 | 317.91 ± 105.40 | NS |
| 4 weeks | 246.50 ± 119.06 | 343.74 ± 162.27 | NS |
| Changes | -67.29 (21.44%) | 25.83 (7.51%) | $p$<0.05 |

*Note:* Control groups: without treatment with PSPOM ($n$=10); Test groups: treated with PSPOM, (n=15); BMI: Body Mass Index NS: Not significant at α=0.05 level by independent $t$-test; Changes: the difference values between baseline and after 4 weeks. $OD_{450}$: optical density at wavelength 450 nm.

**The characteristics of the T2DM respondents and changes in metabolic profile during the 4-week treatment**

The characteristics of the T2DM respondents are presented in Table 2. The ages of respondents were around 55 years old, their weights were between 56.90 and 59.20 kg and the BMI values were 26.18 ± 3.88 and 24.12 ± 3.84 kg/m$^2$, respectively.

The indicators of diabetes such as FBG, HbA1c, and insulin levels are presented in Table 2. The table shows the changes in FBG, HbA1c and insulin level of T2DM control and treated respondents with PSPOM for 4 weeks. Mean FBG levels in both groups were not significantly different at the baseline. However, the means in both groups after 4 weeks show

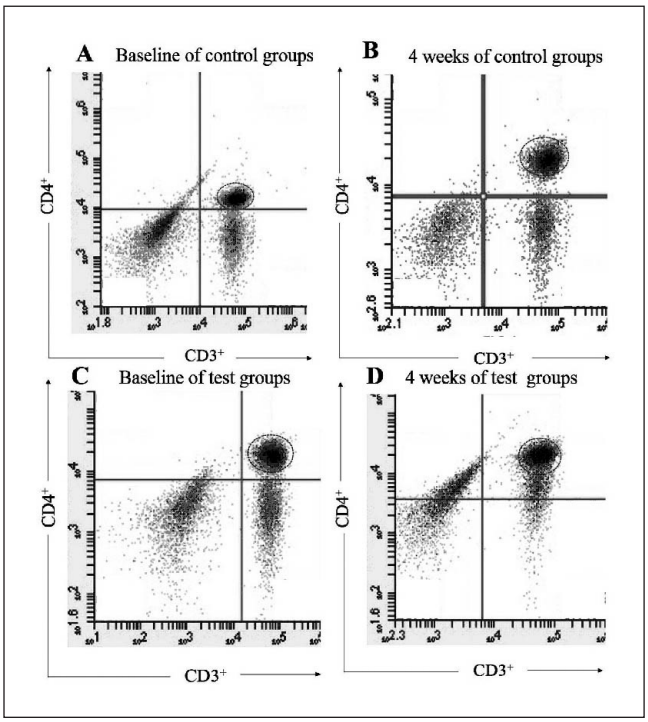


**Figure 2.** The representative of flow cytometric dot plots illustrating the expression (arrow) of CD3+CD4+ T cells before (left) and after (right) intervention of control groups and test groups.

significant decrease ($p$<0.05). The means of HbA1c levels in both groups were significantly different at the baseline. It was also observed that the mean of the HbA1c level of the test group after 4 weeks was significantly lower ($p$<0.05) compared to the control group. The means of insulin levels of test group after 4 weeks were significantly higher than that of insulin levels of control group.

The indicators of immuno-modulator in this study, IL-6 level and $CD4^+$ and $CD8^+$ T cells, are presented in Table 2. This table shows the change in IL-6, $CD4^+$ and $CD8^+$ T cells of the T2DM control and respondents with PSPOM for 4 weeks. The mean of IL-6 levels in both groups was significantly different at the baseline, however, the means in both groups after 4 weeks was not significantly different. After 4 weeks, both groups showed increased levels of IL-6, but the increase in the test group was significantly lower than in the control group ($p$<0.05).

The representative flow cytometric dot plots of the $CD4^+$ and $CD8^+$ T cells in the form of absolute values are shown in Figure 2. The means of $CD4^+$ and $CD8^+$ T cells in both groups did not significantly differ at the baseline. However, after 4 weeks, the means of $CD4^+$ T cells of both groups were significantly different ($p$<0.05) while the means of $CD8^+$ T cells of both groups did not significantly differ ($p$<0.05). After 4 weeks, $CD4^+$ T cells in the test group increased dramatically (116.82 cells/μL), while $CD4^+$ T cells in control group decreased slightly (16.67 cell/$\mu$L). $CD8^+$ T cells in the test group increased slightly (25.83 cells/$\mu$L), while $CD8^+$ T

cells in control group decreased drastically (67.29 cell/$\mu$L) after 4 weeks.

## DISCUSSION

Black soybean (*Glycine soja* (L) *Merrit*) has many benefits due to its good nutritional values such as high in protein and dietary fibres, substantial amounts of bioactive compounds, small amounts of saturated fat, very low glycemic index and absence of cholesterol (Ciabotti *et al*., 2016). The nutritional values of purple soymilk from black soybean in the present study are almost similar to the nutritional values of yellow soymilk. The purple soymilk of our study had moisture, protein, carbohydrate-by difference, fat, and ash of 94.69 ± 0.04, 2.76 ± 0.13, 1.27 ± 0.10, 1.17 ± 0.06, and 0.12 ± 0.08% respectively, while yellow soymilk had moisture, protein, carbohydrate, fat and ash content of 92.02-93.29, 2.23-3.05, 1.99-2.78, 1.60-1.94 and 0.24-0.32%, respectively (Tunde-Akintunde & Souley, 2009). The protein of black soybean had good amino acid score and high digestibility which are important for human nutrient consumption (Ciabotti *et al*., 2016). Zakaria *et al*. (2016) reported that the undigestible carbohydrate in purple soymilk contributes to the very low glycemic index (GI) value of this product. Previous studies of black soybean showed that black soybean had active compounds such as isoflavones, flavonoids, γ-tocopherol, and anthocyanins (Jeng *et al*., 2010). These compounds had pharmaceutical effects and contributed to the antioxidant properties of purple soymilk (Zakaria *et al*., 2016).

The total flavonoid of the purple soymilk in this study was 169.92±1.86 mg/100 g (Meriyanti, 2015) and was higher than that of yellow soymilk (136 mg/100 g, Carrão-Panizzi *et al*., 1999). Free daidzein and genistein flavonoid values were 720.96 and 27.05 $\mu$g/g, respectively (Zuhri, 2015), while the anthocyanin concentration of the purple soymilk in this study was 3.566 mg C-3-G equivalents, which comes only from the seed hull (Zakaria *et al*., 2016).

The BMI values in both groups were between 24.12 and 26.18 kg/m$^2$ which are in the category of overweight (≥24.90 kg/m$^2$), and pre-obese (25.00–29.90 kg/m$^2$) (WHO, 2016). A strong correlation has been established between high BMI value and the development of T2DM. The obesity-induced insulin resistance due to a decrease in insulin-sensitive receptors as the body weight increases has been shown (McArdl *et al*., 2013).

Generally, nutrient intake of the T2DM respondents in the test group was lower than in the control group. The PSPOM consumed by the test group in this study contained protein, fat, and dietary fibres. These components provided longer satiety that may reduce the consumption of other foodstuffs in the test group (Veldhorst *et al*., 2009). Respondent consumption was not controlled because their food pattern was assumed to be not different since they lived in villages with similar living conditions. This similarity in food consumption was strengthened by food recall investigation using questionnaires. The results showed that food consumption was almost the same, only differed in quantity.

In this study, PSPOM in the diets of T2DM respondents significantly reduced FBG and HbA1c levels, while significantly increased the insulin level. These results are similar with those of the T2DM respondents who were treated with soy products. Sedaghat *et al*. (2015) reported that the consumption of 60 grams of soy nuts significantly reduced the FBG and HbA1c levels in T2DM respondents. Shahbazian *et al*. (2006) reported that the consumption of 25 grams of soy protein isoflavones for 3 months significantly reduced the FBG and HbA1c levels in T2DM respondents. It should be noted that isoflavones, a group of flavonoids, could impact insulin action by their well-known

receptor-mediated estrogenic activity. Soy protein and its isoflavones have been reported to significantly increase the insulin level in diabetic post-menopausal women (Jayagopal *et al*., 2002).

Genistein has been reported to be a protective agent for diabetes by regulation of oxidative stress and inflammation as it decreases lipid peroxidation, inhibits cyclooxygenase expression and myeloperoxidase activity as well as reacts with free radicals and neutralises their effects (El-Kordy & Alshahrani, 2015). El-Kordy and Alshahrani (2015) had reported that genistein at 20 mg/kg/day for 4 weeks could improve both glucose and insulin levels in rats with streptozotocin (STZ)-induced islets damage. This compound has a protective effect on pancreatic $\beta$-cells damage, and has the ability to regenerate $\beta$-cells and the capacity to improve serum levels of insulin and glucose.

Hasrini *et al*., (2017) reported that the total carotenoid of CPO microcapsule was 295.24 $\mu$g/g. Ylönen *et al*. (2003) had reported that dietary carotenoids in men and women were inversely associated with FBG concentrations and insulin resistance.

In this study, IL-6 level in T2DM respondents was repressed by PSPOM consumption. Anti-inflammatory activity of PSPOM might be due to its genistein and carotenoid content. Ji *et al.* (2012) reported that soy isoflavone genistein efficiently suppressed overproduction of IL-6 induced by LPS in RAW 264.7 macrophages. Moreover, Bai *et al*. (2005) reported that $\beta$-carotene significantly suppressed lippopolysaccharides (LPS)-induced IL-6 production.

PSPOM product could increase the level of $CD4^+$ and $CD8^+$ T cells. $CD4^+$ is a marker protein on T helper (Th) cells, while $CD8^+$ is a marker protein on T cytotoxic (Tc) cells. The increasing level both of $CD4^+$ and $CD8^+$ T cells is expected to increase immune responses. It is estimated that the combination of isoflavones and carotenoids in PSPOM product may result in stronger immunomodulation. The increase in $CD4^+$ T cells of the group that consumed PSPOM was higher (116.82 cell/$\mu$L blood or 17.18%) than the group which consumed only purple soymilk without CPO microcapsule (58.09 cell/$\mu$L blood or 8.14%) (Zakaria & Hermansyah, pers comm). Klein *et al*. (2002) found that male rat offspring exposed to diets containing genistein during gestation and lactation had higher percentages of $CD4^+CD8^+$ thymocytes, $CD8^+$ splenocytes, and total T cells in the spleen than those not exposed to genistein. Furthermore, the numbers of total $CD4^+$ and $CD8^+$ T cells after the supplementation of CPO in healthy women respondents were higher than in the respondents without CPO supplementation (Waryati, 2012).

## CONCLUSION

The PSPOM product can be recommended for consumption of T2DM respondents. It is rich in functional compounds such as flavonoid, genistein, anthocyanin and carotenoid and has very low glycemic index. This product could decrease the FBG and HbA1c levels, while increase the insulin level which indicates anti-diabetic activities. PSPOM could increase the $CD4^+$ and $CD8^+$ T cells levels and suppresse IL-6 level, indicating immunomodulatory activities.

### Conflict of interest

The authors declare that there is no conflict of interest.

## ACKNOWLEDGEMENTS

The authors are grateful to the Ministry of Research, Technology and Higher Education of the Republic of Indonesia and Kreasi Inovasi Prosana Ltd, Jakarta, for financial support towards this research.

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