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Anti-inflammatory effects of functional milk drink enriched with soya bean sprout protein in breastfeeding mothers

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ABSTRACT

Introduction: Increased metabolism during pregnancy and breastfeeding results in increased oxidative stress among mothers. However, daily intake of foods containing antioxidants can improve antioxidant and inflammatory status. The objectives of this research were to formulate a soya bean sprout protein milk (SSPM) functional drink; to know its protein and isoflavone contents; and its effect on interleukin 6 (IL-6) level in plasma and breast milk (BM) of breastfeeding mothers. **Methods:** The study begun with the production of soya bean sprout protein extract (SSPE), followed by preparing five formulated SSPM, namely SSPE, low-calorie sweetener, fructose, skim milk, and salt. The formulated products were subjected to organoleptic test on a scale of 1 (extremely dislike) to 5 (extremely like). Fifty mothers aged 20-35 years who had a newborn up to six months old, in good health condition, and with informed consent were recruited. They were randomly divided into two groups: group I was fed SSPM for two months, 150 ml/day every morning, and group II was treated as placebo. Data were analysed with one-way analysis and paired sample *t*-test. **Results:** The preferred SSPM composed of 65.0% SSPE, 1.5% low-calorie sweetener, 6.0% fructose, 27.0% skim milk, 0.5% salt, and containing 13.77±0.001% protein and 229.9±0.001mg/g isoflavone content. Two months after intervention, there was a decrease in the level of IL-6 by 82.5% in the plasma (p=0.015) and 68.1% in BM (p<0.05). Body mass index (BMI) decreased from 22.77 to 20.64 kg/m^2 (p=0.019). **Conclusion:** SSPM is a potential anti-inflammatory agent and has health benefits for breastfeeding mothers.

Keywords: Formula of functional drinks, soya bean sprout protein milk, isoflavone, IL-6, BMI

INTRODUCTION

Increased metabolism during pregnancy and breastfeeding may result in oxidative stress in the body (Stuebe & Rich-Edwards, 2009). This condition makes the antioxidant status of breastfeeding mothers unable to compensate for the formation of free radicals. Oxidative stress that occurs in the mother's body during pregnancy is indicated by high levels of free radicals (Winarsi, Sasongko & Purwanto, 2016). Free radical reactivity damages the placental cell membrane, which then impairs placental function (Winarsi, 2007). Besides causing susceptibility towards

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diseases, oxidative stress also affects the growth and development of the baby. Oxidative stress is also exacerbated by the increase in body weight (BW) (Huang *et al.*, 2015). According to Vincent, Morgan & Vincent (2017), obesity is characterised by increased dietary fat intake, increased fat storage, and excessive intracellular triglycerides, and dyslipidemia. Oxidative stress may be due to the metabolic impact of intracellular triglycerides (Bakker *et al.*, 2000). For example, by suppressing the mitochondrial adenine nucleotide transporter, excessive triglycerides may increase $O_2^{\bullet-}$ production within the mitochondrial electron transport chain, and this decreases intra-mitochondrial adenine diphosphate. Electrons then accumulate within the electron transport chain and react with adjacent O_2 to form radical $O_2^{\bullet-}$.

On the other hand, the increase in BW also disrupts the physical appearance of mothers, especially those who have to return to work in offices after their maternity leave period is over. Therefore, they will strive to lose weight without interfering with breast milk production. Breast milk is the best nutrient for babies aged 0-6 months old because the nutritional content is suitable for the growth of babies. Daily intake of foods containing sufficient antioxidants can improve the antioxidant status of the mother and baby. Therefore, it is important to enhance the antioxidant status of breastfeeding mothers with antioxidant-rich foods.

According to Hu *et al.* (2013), soya bean protein is capable of reducing BW more than carbohydrate. Mikkelsen, Toubro & Astrup (2000) stated that soya protein is similar to animal protein in terms of energy expenditure (EE). This is because protein is thermogenic and makes one feels full. Protein stimulates pancreatic beta cells for insulin secretion (Newsholme *et al.*, 2005) and induces pancreatic alpha cells for glucagon secretion (Calbet & MacLean, 2002). Glucagon regulation prevents against hypoglycaemia as glucagon stimulates glucose output by increasing glycogenolysis and gluconeogenesis in the liver (Jiang & Zhang, 2003). Glucagon also plays a role in lipolysis and amino acid metabolism, both of which have different EEs. Glucagon increases EE by stimulating gluconeogenesis and protein oxidation. Thus, adequate protein intake can provide energy faster, namely by glycogenolysis, gluconeogenesis, and protein oxidation, thus promoting weight loss. Regarding energy expenditure, it is known that protein has the highest and longest-lasting thermal effect (20-30%), followed by carbohydrates (5-15%) and fat (3%) (Steinert *et al.*, 2011). Studies that measured diet-induced thermogenesis (DIT) for more than 24 hours (Westerterp *et al.*, 1999), more than a few hours (Johnston, Day & Swan, 2002), or after one protein preload (Luscombe *et al.*, 2003) all suggested that a diet that is higher in protein has a greater effect on energy expenditure, than does a diet that is lower in protein. These findings showed that protein has a lower energy efficiency than carbohydrates or fats.

However, soya bean milk is still less favoured by many people due to its beany flavour. One way to reduce this flavour is through germination. Germinated soya beans are known to be rich in antioxidants, as reflected in its higher protein, amino acids, and isoflavones content compared to non-sprout soya beans (Winarsi, Purwanto & Dwiyanti, 2010). It is stated that the protein and isoflavone contents of soya bean increase as it is germinated. Isoflavones with antioxidant characteristics are reported to have an anti-inflammatory potential, which can reduce plasma interleukin 6 (IL-6) level (Yu *et al.*, 2016). But so far, there is no information whether or not isoflavones in soya bean sprout protein milk (SSPM) can reduce levels of IL-6 in the plasma and breast milk of breastfeeding mothers.

According to Winarsi *et al.* (2010), isoflavones in germinated soya beans have higher antioxidant activity than those non-germinated. It is reported that soya bean protein composes of several peptides with potential as antioxidant agents. The high content of protein and isoflavones, supported by low beany flavour, makes soya bean sprout a good candidate for functional food. However, to date, no formula of SSPM that is preferred by the public has been reported. Besides, the protein and isoflavone contents of SSPM, as well as its effect on IL-6 levels and BW of breastfeeding mothers are not yet known. This study aimed to obtain the SSPM formula which is most preferred by the community. Analysis of protein and isoflavone contents were also carried out on the preferred SSPM. Finally, the effects of SSPM administration on IL-6 levels in plasma and breast milk, and body mass index (BMI) of nursing mothers were studied.

MATERIALS AND METHODS

Production of soya bean sprout protein extract (SSPE)

SSPE was obtained by extracting protein from germinated soya bean of the Slamet variety using physiological sodium chloride (NaCl) and phosphate buffer. SSPE yielded soft white creamy powder. Soya bean (Slamet variety) seeds were thoroughly cleaned, placed on a bamboo tray, and stored in a rather humid room. The soya bean seeds were frequently splashed with water to germinate them. The germinated soya beans were added with physiological NaCl (1:5), stirred for 15 minutes, and then extracted in a blender to form a porridge. The porridge was added with phosphate buffer to reach pH 5 and centrifuged at 10.000 rpm at 4^{o}C for 20 minutes. The pellet was separated from the supernatant, oven-dried at 50-70^{o}C, then crushed in the blender to obtain soft white creamy SSPE powder (Winarsi & Purwanto, 2015).

Formulation of functional drinks enriched with SSPE

To obtain a favourable and highly nutritious functional drink enriched with SSPE that can improve bodily functions, five formulas which composed of SSPE, sweetener (Tropicana® sugar), fructose, skim milk, and salt were made (Table 1). Ingredients were mixed and stirred until homogenous. The most favourable formula was selected from the five through an organoleptic test.

Table 1. SSPE proportion in several formulas of beverage enriched with soya bean sprout protein extract

Ingredients (%)	*Formula code*				
	A	*B*	*C*	*D*	*E*
SSPE	60.0	65.0	70.0	75.0	80.0
Tropicana® sugar	1.5	1.5	1.5	1.5	1.5
Fructose	6.0	6.0	6.0	6.0	6.0
Skimmed milk	32.0	27.0	22.0	17.0	12.0
Salt	0.5	0.5	0.5	0.5	0.5
Total	100.0	100.0	100.0	100.0	100.0

Note: SSPE, soya bean sprout protein extract

Sensory test of functional drink enriched with SSPE

Sensory test (hedonic test) was performed to investigate consumer acceptability on colour, flavour, and taste of the functional drink, which was presented in an infused formula (25g of formula plus 125ml of hot water and stirred until homogeneous). The panelists were 55 individuals, consisting of students, laboratory workers, and university employees in the researcher's workplace. They were inquired to express their personal opinions of like or dislike on a hedonic scale of 1-5, where 1=extremely dislike, 2=moderately dislike, 3=neutral, 4=moderately like, and 5=extremely like. To verify the acceptability of each product and to standardise the evaluation of sensory attribute, an acceptable factor (AF) was determined according to the Dutcosky (1996) formula as follows:

AF= A. 100. B–1 ....(1)

notes: A, mean of each attribute; B, the maximum mean of each attribute

The most preferred formula was subjected to the proximate and isoflavones content analysis test. The analysis for protein and fat was carried out by the Association of Official Analytical Chemists (AOAC) official methods of analysis (AOAC International, 2000), while isoflavones content was determined by the Küçükboyac *et al.* (2013) method.

This research was conducted in a randomised clinical trial double-blind method. Authors and subjects did not know which product was given. Ethical clearance for this research was approved by the ethics committee of the Medical Faculty of Diponegoro University, Semarang, Indonesia.

Research subjects and intervention

Fifty lactating mothers aged between 20-35 years old, having either a newborn or older baby (up to six months old), in good health condition, lived in Purwokerto, Central Java, Indonesia, and agreed to sign an informed consent were asked to be respondents.

All subjects were randomly divided into two groups (25 each). During the time of this research, group I was given SSPM in their daily diet, while group II was given a placebo. Both groups consumed 150ml/day of each of their drinks for two consecutive months. The placebo and SSPM were ready-to-drinks, given every morning between 6:00 to 8:00 a.m., delivered by the enumerator to the subject's home. In this study, the enumerator witnessed the subjects drinking the given product. Besides, subjects also recalled food consumption eight times - four times on weekdays, and four times on holidays. If any adverse events occurred on the subject, the subject was then excluded and handled by a designated doctor.

Sample of blood and breast milk

Blood and breast milk samples were taken three times at zero (before intervention), one and two months after intervention. All samples were taken in the morning, before breakfast. Sampling was done by taking 3ml of blood intravenously using a venoject tube containing ethylenediaminetetraacetic acid (EDTA)-10%, and then centrifuged at 0.503 x g for ten minutes to separate the plasma.

Breast milk sample as much as 3ml was taken manually by the subjects. The sample was also taken in the morning before breakfast, and then centrifuged at 0.503 x g for ten minutes at 4°C. The lipid layer on the surface was used for sample testing.

Determination of IL-6 levels in plasma and breast milk

IL-6 levels in plasma and breast milk were measured using specific kits, i.e.

Biovision Assay and Uscn Life Science Inc. Elisa reader Labotron LB-6200 was used to read the data.

Body weight measured as BMI

BMI is a ratio of weight in kilograms to height in meter squared. Subject's weight was weighed on a balance scale stampede Camry 0-130kg, while height was measured using a 0-200 cm scale microtoise. Weight and height measurements were performed in conjunction with the time of blood sampling.

Data analysis

The research data were analysed by one-way analysis of variance with repeated measures and by paired sample *t*-test. Differences between means were considered significant at $p<0.05$.

RESULTS

The SSPE yield was 28.6±0.003% (Winarsi *et al.*, 2010). Organoleptic or sensory test on colour, flavour, and taste of all the five infused formulas were expressed as average value±standard devation (SD) and acceptability factor (AF), as presented in Table 2. The hedonic test revealed that the most preferred formula based on colour and taste of the product was formula B, a SSPM drink that contained 65.0% SSPE, 1.5% low-calorie sweetener, 6.0% fructose, 27.0% skim milk, and 0.5% salt. The panelists generally gave a high score to this SSPM formula with 65.0% SSPE, followed by 75.0%, 60.0%, 80.0%, and 70.0%. The sensory test revealed that the highest preference was taste, followed by the colour and flavour of SSPM. Functional foods are not merely about deliciousness or high sensory scores, but must be nutritious and functional for the body. The nutritional value of the selected formula was observed based on the analysis of proximate and isoflavones (Table 3).

Traditional soya food products like bean curd and soya protein isolates generally contain 0.25-40.00mg isoflavones, while soya milk has 0.1-2.0mg/g soya protein. This research reported that the content of isoflavones in SSPM was as much as 229.9mg/g protein, much higher than that of traditional products. Isoflavone is a prevalent substance in soya, and often utilised in oestrogenic and antioxidant supplements (Winarsi *et al.*, 2016). As an oestrogenic supplement, isoflavone is

Table 2. Acceptance (average values±SD) and acceptability factors (AF) for formulas of milk beverage enriched with SSPE (*n*=55)

Preference Score	*Formula code*[†]				
	A	*B*	*C*	*D*	*E*
Colour	$3.6±1.1^{a}$ (100)	$3.6±0.8^{a}$ (100)	$3.3±1.2^{a}$ (91.7)	$3.5±0.8^{a}$ (97.2)	$3.3±0.8^{a}$ (91.7)
Flavour	$3.2±1.1^{a}$ (100)	$3.2±0.8^{a}$ (100)	$2.8±0.9^{a}$ (87.5)	$3.2±0.7^{a}$ (100)	$3.2±0.8^{a}$ (100)
Taste	$3.6±0.9^{a}$ (97.3)	$3.7±0.9^{a}$ (100)	$3.2±1.0^{b}$ (86.5)	$3.6±0.8^{a}$ (97.3)	$3.6±1.0^{a}$ (97.3)

[†]A is a formula with 60.0% SSPE; B is a formula with 65.0% SSPE; C is a formula with 70.0% SSPE; D is a formula with 75.0% SSPE; E is a formula with 80.0% SSPE
Values from the same row with different letters show significant differences at $p=0.05$
Score: 1=extremely dislike, 2=moderately dislike, 3=is neutral, 4=is moderately like, 5=is extremely like; SSPE, soya bean sprout protein extract

Table 3. Proximate and isoflavone content of functional milk beverage enriched with SSPE

Components	*Milk enriched with SSPE*	*Soya Milk*	*Placebo*† *(%)*
Water (%)	80.1±0.0^a	85.2±0.0^a	81.4±0.0^a
Ash (%)	0.07±0.0^a	6.2±0.0^b	0.1±0.0^a
Protein (%)	13.8±0.0^a	3.6±0.0^b	11.5±0.0^a
Lipid (%)	0.9±0.0^a	2.0±0.0^b	0.8±0.0^a
Carbohydrate (%)	5.1±0.0^a	2.9±0.0^b	6.2±0.0^a
Isoflavone (mg/g protein)	230.0±0.0^a	60.1±0.0^b	

†Placebo was powdered cow's milk without SSPE
Note: n=3
Numbers with similar letter show non-significant differences (p>0.05)

bound with the oestrogen receptor in the body, which affects the body's dependency on oestrogen. As an antioxidant, isoflavone has several medical functions such as anticancer (Goodman *et al*., 2011), anti-diabetes, body weight control, anti-atherosclerosis (Winarsi *et al*., 2016), and anti-inflammatory (Yu *et al*., 2016). Accordingly, besides protein, lipid, and carbohydrate, SSPM is also rich in isoflavones that exceed the amount contained in non-germinated soya milk. With that, it was given to breastfeeding mothers to find out its effects on IL-6 levels in plasma and breast milk.

At baseline, the level of IL-6 of lactating mothers was 18.8pg/mL. This value was higher than the plasma levels in non-pregnant or non-breastfeeding healthy women (1.0-0.2pg/mL), but lower than in women with type 2 diabetes mellitus with obesity (22.0pg/mL). High levels of IL-6 at baseline are a natural compensation in the body of breastfeeding mothers who have new babies. In postpartum conditions, the levels of inflammatory cytokines are higher as a result of activated inflammatory cells. Esposito *et al.* (2004) suggested that normally, IL-6 levels are very low, but the level is increased as the body experiences infection, trauma, ageing, and stress. Breastfeeding mothers experience stress with their conditions as new mothers. However, IL-6 plasma level decreased significantly from 10.2 to 1.8pg/mL (p=0.015), after two months of SSPM consumption, possibly due to the isoflavone content in the drink (Figure 1).

Lactating mother's weight expressed as BMI was initially 22.8kg/m^2, which did not indicate obesity. However, taking into account the pre-pregnancy BMI which was 19.3kg/m^2, it can be said that the BMI of subjects increased significantly (p<0.004) from pregnancy to lactation. There was a decrease in BMI among lactating mothers, from 22.8 to 20.6kg/m^2 (p<0.006) in the group who consumed SSPM for two months, while the control group did not show any significant changes (Figure 2).

DISCUSSION

SSPE yield was 28.6±0.003%, relatively higher than non-germinated soya (22.2±0.04%) (Winarsi *et al*., 2010). The protein content in germinated soya powder and non-germinated soya powder were 42.0% and 35.6%, respectively. The content of germinated soya protein increased compared to that in soya powder, probably due to

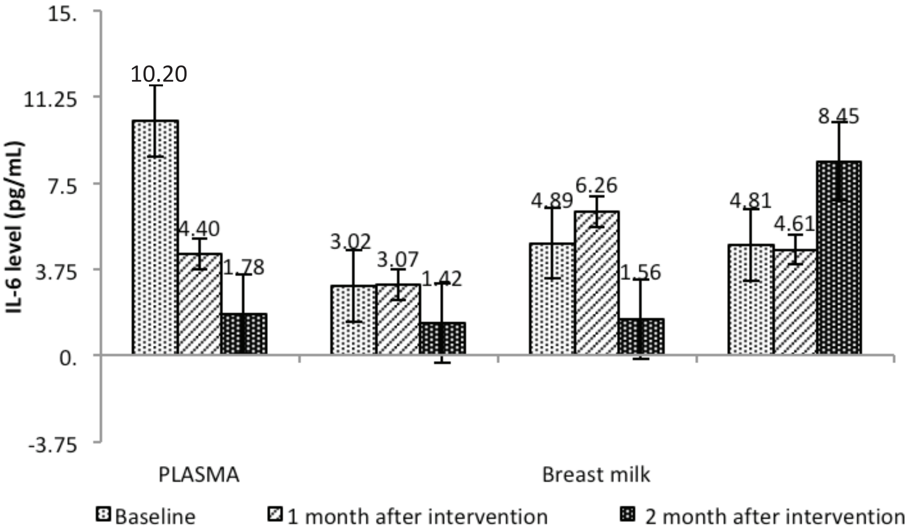

Figure 1. IL-6 level of lactating mothers who consumed SSPM
Notes: SSPM, the group consuming soya bean sprout protein milk; Plac, group consuming placebo; numbers with similar letters show a non-significant difference (*p*>0.05)

the germination process that increased total soluble solid, thereby increasing yield. Furthermore, SSPE was rich in isoflavones (39.1ppm) compared to soya protein isolates which only had 26.7ppm. High SSPE yield, protein, and isoflavones enabled the powder to be used as a functional food ingredient. This finding revealed that SSPE obtained from germinated soya can be formulated

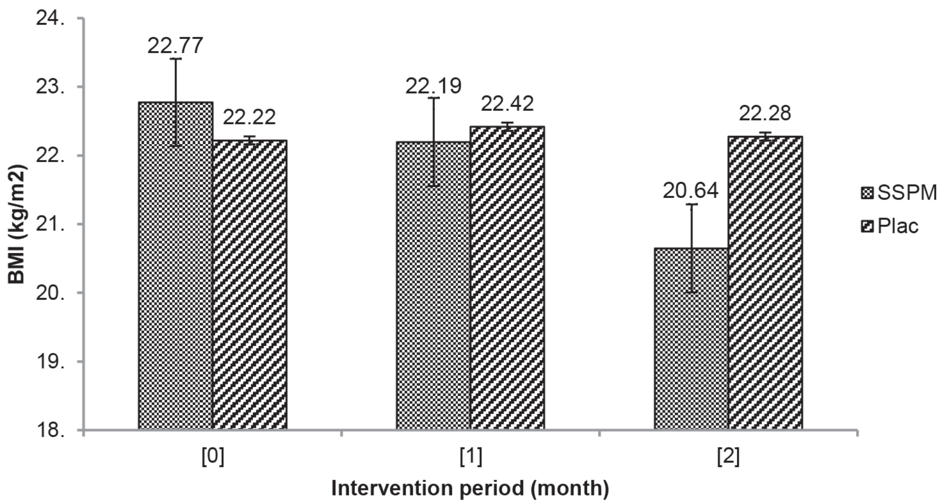

Figure 2. Effect of SSPM on BMI of lactating mothers
Notes: SSPM, group consuming soya bean sprout protein milk; Plac, group consuming placebo; 0, 1, and 2, intervention period in month; BMI, body mass index, n = 25; $P < 0.05$; numbers with similar letter show non-significant differences (*p*>0.05)

into functional food products. To date, SSPE has not been commercialised. This finding therefore provides an opportunity for the mass production of SSPE.

The sensory of SSPM

Regarding AF value, formulas A and B had a maximum score (100), and were therefore selected. It was likely that both formulas had an attractive colour. A nutritious and delicious food product will be less preferable if the colour is dull or deviant from the common shades. Wilson *et al.* (2017) explained that colour, among other factors such as taste, frequently serves as a main consideration and in some cases, significantly determines the selection of a product. Food or beverage colour is due to naturally formed pigments from plant or caramelisation, with dark colour due to Maillard reaction, enzyme oxidation, and additional colourant. The colour of a product is a contribution of water-soluble substances, which in SSPM were protein, carbohydrate, vitamins B1 and B2.

According to Topin *et al.* (2014), flavour is the response of our smelling sense in the nasal cavity. Flavour is among the determining properties of the deliciousness in foods. Flavour is also defined as something detected by the smelling sense, and therefore essential to determine food deliciousness. Flavour is derived from chemical reactions and interaction with other ingredients. The detectable beany flavour in SSPM product is due to the lipoxygenase enzyme in soya that can hydrolyse polyunsaturated fatty acid and produces volatile compounds (Peng *et al.*, 2016). The flavours of these products were not different among formulas (p=0.22), and all AF was 100, except for formula C (87.5), therefore the selected formulas were A, B, D, or E. It was likely that lipoxygenase activity was inhibited due to germination, milling and infusion processes, so the odd flavour was lessened and the preferable flavour released. Preference scores in all of the products across formulas were not significantly different too (p=0.25), except for formula C, while the highest AF score was found in formula B, and so it was selected.

Taste is a crucial component in food products. A food can release favourable taste depending on its substances. Food ingredients are generally composed of not one, but a combination of various tastes that generate a whole taste of food. Factors like consistency of food ingredients affect food taste. The change in flavour or taste of food ingredients is usually more complex than that in colour (Spence *et al.*, 2010). Taste is related to the response of chemical stimulation by the sense of taste (tongue) as an interaction between flavour, taste, and texture of food. Ammari & Schroen (2018) stated that taste is contributed by some factors namely chemical substances that can release different flavours. Acidic intensity depends on H+ ion production, whereby sweet flavour is due to aliphatic compound, and bitter taste is due to alkaloids. Temperature affects one's tasting ability to detect taste stimulation. Concerning concentration, each person has a lowest limit to taste a product concentration. The interaction among components that react to the primary taste component also affects the flavour of a product.

Effect of isoflavones and protein content of SSPM on IL-6 levels and BMI of breastfeeding mothers

The protein content in SSPM was 13.8±0.001%, higher than that in non-germinated soya milk (3.6±0.002%). The increase in protein content is supported by the finding of Narsih, Yunianta & Harijono (2012) on germinated sorghum. During germination, the activity of the protease enzyme (involved in the degradation of peptide component to amino acids) increases, and the amount of protein will increase. This increase is due to the presence of protein hydrolysis, as well as the result of protease enzyme activity during germination of the seeds. In this case, protease enzymes break down the peptide bonds in proteins and produce amino acids, subsequently increasing protein content. The increase in protein content during seed germination will increase the mobilisation of nitrogen-fixing and thus improve the quality of proteins used for the development of young plants.

In this study, the highest protein content in milk enriched with SSPE was 13.8±0.001%. It is mentioned that the highest amino acid content in soya protein is glutamic acid (190.11 mg/g) (Liu, 1999). There is a possibility that glutamic acid content was also the highest in our SSPE product, although its level was not analysed in this study. Glutamic acid gives an umami taste, which is a delicious savoury taste that many like. Besides glutamic acid, soya protein also contains tryptophan. The body converts these amino acids into simple molecules called 5-hydroxytryptophan (van den Oord & van Wassenar, 1997). This molecule is an important raw material in the synthesis of serotonin, melatonin, and vitamin B6. Serotonin is a chemical that functions to send signals between nerve cells, regulates mood, and influences behaviour. Meanwhile, melatonin plays a role in regulating the sleep cycle, and vitamin B6 is needed to play a role in the formation of energy. Thus, tryptophan creates a positive mood, and makes panelists like milk enriched with SSPE.

During the germination process, all soya components including protein, carbohydrate and lipid are hydrolysed. Strong component bonds start to detach that allow digestion. Germination can also increase protein digestibility due to reserve protein degradation. Some components in germinated soya protein that play a role in decreasing sugar levels are binding amino acids and isoflavones. Three poly-chained amino acids such as leucine, valine, and isoleucine prevalent in germinated soya protein are crucial in the metabolism process (Wolfe, 2017). When consumed, these amino acids are directly detected in plasma and peripheral tissues including skeletal muscles and adipose tissues. Therefore, the increasing level of poly-chained amino acids accelerates catabolism in peripheral tissues. Poly-chained amino acid degradation in skeletal muscles is related to alanine and glutamine production that maintain glucose homeostasis.

Winarsi *et al.* (2016) stated that germinated soya protein contains amino acids that trigger higher insulin excretion than non-germinated soya protein. Amino acids triggering insulin secretion are arginine, lysine, phenylalanine, alanine, leucine, and isoleucine, which are prevalent in soya protein, including germinated soya protein. Moreover, germinated soya beans contain amino acids glycine and arginine that control insulin hormone. Accordingly, SSPM is very suitable for a diabetic person.

Soya protein diet can lower triglyceride levels mainly in the liver. Germinated soya protein is likely to lower triglycerides since its protein can block activities of the hepatic lipogenic enzyme, particularly glucose 6-phosphate dehydrogenase and synthesised fatty acids, as well as acetyl-CoA carboxylase (ACC). ACC is an enzyme that crystallizes carboxylated acetyl-CoA into malonyl-CoA. However, the inhibited activity decreases malonyl-CoA, and triglycerides are formed. The decreasing triglyceride is correlated with the increasing activities of several skeletal muscle enzymes that play a role in fatty acid oxidation including carnitine palmitoyltransferase and beta-hydroxy acyl-CoA, medium-chain acyl-CoA oxidase and acyl-CoA dehydrogenase. Therefore, the protein-rich SSPM is expected to reduce triglycerides or liver lipids.

The fat content of SSPM was 0.9±0.001%, lower than that of soya milk (2.0±0.002%). Similar finding was reported by Narsih *et al.* (2012) on germinated sorghum, whereby during germination, lipase activity increases, thus degrading soya fat into glycerol and fatty acids. Glycerol and fatty acids are water-soluble, thereby diffused into cell tissues. Accordingly, germination increased the hydrolysis of complex organic compounds in insoluble soya bean into less complex and water-soluble organic compounds.

Ash content is the parameter of inorganic matter value in a product. The higher the level, the more inorganic matters contained in a product. The components of inorganic matter vary in type or amount. Ash content of SSPM was 0.1±0.002%, lower than that in soya milk (6.2±0.001%). A similar finding was reported by Chaudhary & Vyas (2014) that millet germination-based premixes reduced ash content, while soaking time increased the loss of minerals used for growth of the sprout fine root since ash content was depleted. Ash content can be a parameter of the nutritional value of ingredients - the lower the ash content, the lesser the minerals. Therefore, SSPM is suitable for diabetic patients who suffer from kidney disorders with limited mineral intake.

The level of carbohydrate increased in germinated soya protein milk. Carbohydrate in this research likely included fibre. Warle *et al.* (2015) stated that the increasing carbohydrate level is derived from the outer layer of soya. Crude fibre commonly decreases in soaked mung bean, but conversely, increases in rice and soya. It is made clear that the soaking stage (before germination) affects the level of crude fibre, but not carbohydrates.

Germination changes the biochemical composition of grains including carbohydrates. During germination, the activity of α-amylase and β-amylase increase. Germination is responsible to increase amylose but decreases amylopectin. The germinated cereal grains cause extensive changes in the structure and composition of macromolecule substances. High carbohydrate level as fibre in SSPM is significantly beneficial for diabetic, cardiac, and hypercholesterolemic patients (Winarsi *et al.*, 2016).

During the postpartum period, there is an increase in inflammatory responses in the serum, which reflects the activation of the inflammatory response system (Christian & Porter, 2018). This condition is related to several factors such as physiological stress, including changes in life, lack of social support, and the possibility of illness in infants. Sleeping disorders and pain are physical stress that are common among new mothers, which also increases the risk of depression. Physical and psychosocial stresses increase inflammation, which is reflected by high levels of IL-6.

In general, mothers who have new babies feel happier. However, there is a change in the quantity and quality of sleep. Disrupted sleep periods can damage physical health and adversely affect the work of the immune system (Chattu *et al.*, 2019). Sleep disturbance generally occurs in the postpartum period, thereby increasing levels of night cortisol, glucose, and insulin resistance.

During the postpartum period, mothers generally experience increased level of stress. Breastfeeding their babies make them calmer, so the response to stress is reduced. Breast milk is an ideal nutrition for infants and is sufficient for the optimal growth of babies in the first six months (Motee & Jeewon, 2014). Breastfeeding is important for the psychological health of postpartum mothers. Several studies have shown that breastfeeding can protect the body against type 1 diabetes, multiple sclerosis, and rheumatoid arthritis. Le Doare *et al.* (2017) revealed the role of IL-6 and transforming growth factor beta (TGF-β) in the emergence of autoimmune diseases in infants because these cytokines are found in baby serum. IL-6 is a multifunctional cytokine that regulates immune responses and acute phase reactions. High levels of IL-6 indicate the pathogenesis of several degenerative diseases, even increasing the risk of myocardial infarction in future, so the levels must be suppressed.

Epidemiological studies explain that soya isoflavones have anti-inflammatory activity in cardiovascular patients. Some researchers even reported a decrease in inflammatory cytokines by isoflavone-rich soya-based food products (Yu *et al.*, 2016; Ferguson *et al.*, 2014). Winarsi *et al.* (2010) reported that in soya bean sprouts, the content of isoflavones was higher than in non-sprout soya beans. Genistein, which is known to be excessive in soya isoflavones, is a tyrosine kinase inhibitor. This enzyme affects the signalling pathway of immune cells, both innate and adaptive immune responses. Thus, these isoflavones suppress IL-6 levels through improved immune response, so that immune cells are no longer activated to produce more cytokines. It was also reported that low-level isoflavones can bind with β-oestrogen receptors and then activate and modulate peroxisomal proliferator-activated receptor-γ (PPAR-γ) (Yu *et al.*, 2016). PPAR-γ is an inflammatory control pathway. On the other hand, it is possible that the lunasin peptide, a result of the hydrolysis of soya bean sprout proteins, is also potentially an anti-inflammatory agent. Anti-inflammation may be an important intervention strategy for the prevention and treatment of cancer (Rayburn, Ezell & Zhang, 2009). Lunasin can be an agent for cancer inhibitor and therapy. The peptide blocks or reduces the NF-kappa-B inflammatory marker, thereby reducing IL-6 levels.

IL-6 levels in breast milk initially amounted to 4.85pg/mL. IL-6 levels can increase up to three times in depressed women. In general, having a baby and breastfeeding are happy times for every family, but because almost all activities change, it could result in high levels of stress, which is reflected in high levels of IL-6. After two months of consuming SSPM, IL-6 levels in breast milk decreased from 4.89 to 1.56 pg/mL, but the decrease was not significant (p= 0.44) (Figure 1). Even so, the levels in breast milk were not different from those in plasma (p>0.05).

Germinated soya milk gives strong benefits to both lactating mothers and their babies, because breast milk is the main food compound in the early phase of a baby's life. Lactating mothers feel happy that they could provide sufficient food supply to their baby through breast milk. Factually, the baby who got SSPM slept much longer (1.85 hour) than those from the placebo group (1.25 hour). Based on these findings, it could be assumed that the treated babies got enough milk and so felt safe due to breast milk from his/her mother.

In this study, it was proven that SSPM was able to reduce BMI of breastfeeding mothers. There are several possible causes for the decline in breastfeeding mothers' BMI. SSPM, which protein is known to be rich, gives a sense of satiety, thus eliminating a sense of wanting to eat again, and suppresses subsequent energy intake. Ortinau *et al.* (2014) reported that a group on a high-protein diet produced longer satiety than those on a diet low in protein, thus suppressing following energy intakes. This occurs because the body does not store protein, so protein is immediately metabolised to energy. The formation of energy is identical to the process of re-oxygenation, which illustrates the improved condition of hypoxia, which in turn decreases the release of IL-6 from adipose cells.

The number of lipids in women is higher than in men, because they have more subcutaneous fat than men, while men contain more abdominal fat (visceral) (Wu *et al.*, 2009). The diversity of depot fat raises the expression of different genes, for example, visceral lipid (men) produces more angiotensin, IL-6, and plasminogen activator inhibitor-1, but lesser leptin and adiponectin. The opposite is true in women who have a subcutaneous fat depot, where levels of IL-6 is lower than men. Thus, SSPM was able to suppress weight, as well as IL-6 levels of breastfeeding mothers with different mechanisms played by isoflavones, peptides, and proteins.

CONCLUSION

The preferred SSPM formula composed of 65.0% SSPE, 1.5% low-calorie sweetener, 6.0% fructose, 27.0% skim milk, and 0.5% salt. The product is thick creamy white, rich in protein (13.8±0.001%) and isoflavones (229.9±0.001 mg/g), and conversely low in fat. SSPM given to lactating mothers for two consecutive months saw a decrease in IL-6 level of the plasma by 82.5%, as well as in breast milk by 68.1%. Aside from being an anti-inflammatory, SSPM was also able to reduce the BMI of breastfeeding mothers by 9.4%, to achieve a normal BMI within two months. This product is fit to be consumed by lactating mothers since it can be used as a source of antioxidants, anti-inflammatory, weight loss, and to provide them with sufficient amount of milk for their babies. It is possible that this product can also be potentially beneficial for obese men and adolescents.

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Authors' contributions

HW, principal investigator, conducted the study, data analysis and interpretation, prepared the draft of the manuscript and reviewed the manuscript; AY, conceptualised and designed the study, led the data collection, advised on the data analysis and interpretation, assisted in drafting of the manuscript and reviewed the manuscript; GRR, led the data collection, advised on the data analysis and interpretation and reviewed the manuscript.

Conflict of interest

The authors report no conflict of interest to disclose in this work.

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