Volume 15, Number 1, March 2020

ISSN 1978-1059
EISSN 2407-0920

JURNAL GIZI DAN PANGAN
JOURNAL OF NUTRITION AND FOOD

Articles

Published by:

Food and Nutrition Society of Indonesia

Department of Community Nutrition, Faculty of Human Ecology, IPB

Available online: http://journal.ipb.ac.id/index.php/jgizipangan ***J. Gizi Pangan, March 2020, 15(1):1-10***
Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019 *DOI: 10.25182/jgp.2020.15.1.1-10*
ISSN 1978-1059 EISSN 2407-0920

A Supplementary Protein Food for Pregnant Women with Chronic Energy Deficiency to Improve Fetal Growth

Fajria Saliha Puspita Prameswari[1], Sri Anna Marliyati[1*], Mira Dewi[1]
[1]Department of Community Nutrition, Faculty of Human Ecology,
IPB University, Bogor, 16680, Indonesia

ABSTRACT

The aimed to analyze the effect of protein-sourced supplementary foods for pregnant women with CED to improve fetal growth. The pre-post study test involved 44 pregnant women with a Mid Upper Arm Circumference (MUAC) of <23.5cm in Bogor City, West Java. The subjects were divided into two groups, first was the PG group, consist of pregnant women who received the protein-sourced supplementary foods. The second was the PCG group where the pregnant women recieved the standard government supplementary food (GSF) for eight weeks. Data regarding the fetal growth was obtained using USG, the inidactor measured were the femur length and estimated fetal weight. The increase of femur length and estimated fetal weight were used as the fetal growth parameters. Analysis of Covariance was applied to obtain the effect of intervention by controlling other covariate variables. The result showed that the improvement of estimated fetal weight (EFW) and femur length (FL) in both groups were similar ($p>0.05$). Despite the fact that the PG group showed lower compliance compared to the PCG ($p>0.05$). The findings suggest that our protein-sourced supplementary foods had a similar effect with the GSF on fetal growth of pregnant women with CED.

Keywords: chronic energy deficiency, fetal growth, pregnancy, supplementary food

INTRODUCTION

The anabolic and catabolic processes during pregnancy leads to increase in maternal nutritional need to ensure optimal fetal growth (Brown 2005). Inadequate nutritional intake during pregnancy causes substantial implications and increases maternal and offspring risks of poor outcomes (Wu *et al*. 2012; Black & Heidkamp 2018). Chronic energy deficiency (CED) is a common nutrition problem for pregnant women in the developing countries. In Indonesia, women with a mid-upper arm circumference (MUAC) of below 23.5cm are classified as having CED (Kemenkes RI 2013). In 2007, the prevalence of CED among pregnant women was 13.6% and increased to 24.2% in 2013, however it declined in 2018 to 17.3% (Kemenkes RI 2007; Kemenkes RI 2013; Kemenkes RI 2018a). According to WHO (1995), CED is considered as poor nutritional condition.

The main cause for maternal CED are inadequate intake of energy and protein during pregnancy. About 53.9% and 51.9% of pregnant women in Indonesia were deficient in energy and protein (Kemenkes RI 2018b). While, inadequate nutritional intake during pregnancy leads to fetal growth failure, IUGR (Intrauterine Growth Retardation), low birth weight (LBW), preterm delivery, and birth defects (Wu *et al*. 2012; Black & Heidkamp 2018). The prevalence of newborn with birth length <48 cm had increased from 20.2% in 2013 to 22.7% in 2018 (Kemenkes RI 2013; Kemenkes RI 2018a). Short birth length may result in stunting, a growth failure in children under 5 years. Stunting is considered as public health nutritional problem, since the prevalence of stunting in Indonesia was 30.8% (Kemenkes RI 2018a). Several health problems may occur as the result of stunting and lead to reduction of individual productivity in the future (TNP2K 2017). Stunting prevention starts from the first 1,000 days of life which include nutrition during pregancy.

Maternal supplementary feeding during pregnancy can support the improvement of maternal nutritional status (Dewey 2016). Balanced energy-protein supplementation (protein <25% of total energy in supplement) which given in the second trimester of pregnancy can significantly increase fetal weight, and if given at late pregnancy can improve fetal growth, increase birth weight and birth length, and also reduce the percentage of LBW up to 6%. The

[*]**Corresponding Author:** +628121105760, email: anna_marliyati@yahoo.com

protein content in supplementary food should be at least 12.3% to be efficient on improving fetal growth (Liberato *et al*. 2013). According to Imdad dan Bhutta (2012), balanced energy-protein supplementation is more effective on increasing birth weight and reducing LBW in pregnant women with poor nutritional status than pregnant women with normal nutritional status.
Indonesian national program to fulfill nutritional needs of CED pregnant women has been done since 2016. The Ministry of Health in Republic of Indonesia distributes biscuits as supplementary food for pregnant women with CED (Kemenkes RI 2017). Thus, accessibility to the intervention is still a problem. Several studies about the efficacy of supplementary food CED pregnant women had beed conducted in Indonesia by measuring several indicators, such as hemoglobin levels (Sairuroh 2019), but none of it measure the fetal growth. This study aimed to determine the effect of protein-source supplementary foods given for pregnant women with CED on their fetal growth.

METHODS

Design, location, and time

This research was a pre-post test experimental study which was conducted from December 2018 to April 2019 and took place in 8 Community Health Centers in Bogor City. The research location was chosen based on the highest prevalence of maternal CED in Bogor according to Bogor City Public Health Office. Baseline and endline data collection were conducted in Klinik Medika Utama, Bogor. Ethical approval for this research was issued by the Ethics Committee for Research Involving Human Subjects, IPB University No:129/IT3.KEPMSM-IPB/SK/2018.

Sampling

The subjects of this research were pregnant women in the second trimester of pregnancy. The inclusion criterias were pregnant women aged 16–35 years old, Mid Upper Arm Circumference (MUAC) <23.5 cm, carried singleton pregnancy, and signed the informed consent. While the exclusion criterias were pregnant women whose allergic to eggs or nuts, had high blood pressure (diastolic blood pressure ≥90mmHg) (WHO 2011), had high random blood glucose (≥11.1 mmol/L) (WHO 2013), and suffered from chronic disease.

Screening process was performed before the baseline data collection. Screening process was conducted two times due to inadequate number of subjects for both groups. The first screening process was done to collect subjects for the intervention group (PG) group and 39 women were selected to follow the procedure, however 7 women did not come to baseline data collection, resulting 32 women to continue the procedure. 5 women were excluded from this study because 4 women were pregnant >7 months and 1 woman had anembryonic pregnancy.

Meanwhile, the second screening process was done to collect subjects for the positive control group (PCG) and 25 women were selected to follow the procedure with 22 women continued the procedure since 3 women did not come to baseline data collection. 1 woman was excluded because she was >7 months pregnant. 48 women, with 27 women in PG and 21 women in PCG, were selected to follow the intervention period. Four subjects were dropped-out during the intervention period. 3 women in PG and 1 woman in PCG were dropped-out because they did not consume the intervention products for a week, resulting in 24 women in PG and 20 women in PCG to continue the intervention. Pregnant women with CED belong to critical group, thus treatment should be given, and hence we need the PCG. The sample size calculation was conducted based on the study of Kaseb *et al.* (2002).

Procedure

Screening process was conducted in an eight predetermined community health center. The designated subjects who fulfill the inclusion criteria were asked to come to the location of baseline data collection. Maternal characteristics were collected using questionnaire. Maternal anthropometric data, namely weight, height, and MUAC were measured on digital weight scale, stature meter, and measuring tape respectively. Fetal growth, indicated by femur length (FL) and estimated fetal weight (EFW) was measured with an ultrasonography (USG) and was compared to a standard fetal growth scheme (Kiserud *et al*. 2017). Finger prick blood sample for haemoglobin (Hb) and random blood glucose level collection were performed using different lancets. The Hb was analyzed using HemoCue, while random blood glucose level was analyzed using a Glucometer. Maternal food intakes were

estimated using 1x24 hours food recall which was performed every two weeks during the intervention period. Maternal food intake before the intervention was measured by food frequency questionnaire (FFQ).

Subjects were distributed into two groups, PG and PCG, and were obliged to consume the products, the high protein supplementary food and the government biscuits for 8 weeks. Subjects in PG were given three types of protein-sourced supplementary foods in the form of snackbar (SB), instant powdered-drink (IPD), and instant cream soup (ICS), whereas subjects in PCG were given government supplementary food (GSF) in the form of biscuit. The products were given alternately, with SB to be given solely for the 1st to 3rd week. And the addition of two other products were given as a combination with SB start from the 4th to 8th week. ICS and IPD were never given solely to the subjects, but always given as combination with SB. The average of protein content of the three products was 18.2% and this percentage meets the criteria of balanced energy-protein supplementary food according to Liberato *et al*. (2013). These products are also considered as protein-sourced food since the protein content is >20% of nutrition label recommendation per 100 g products. The nutrient content of each supplementary food are shown in Table 1.

The subjects received new products once a week which were delivered directly to them. PG subjects received 14 packages of products and were requested to consume 2 packages per day, that equal to 100 g of supplemented food. Whereas, PCG subjects received 7 packages of product with 3 pieces of biscuits in each package, and were asked to consume one package per day. Each package of biscuits weighed 60 g.

Tabel 1. Nutrient content of supplemented foods per 100 gram

Nutrient content	Protein-sourced supplementary foods			GSF
	ICS	IPD	SB	
Energy (kkal)	432	423	447	487
Protein (g)	30.2	14.3	15.3	10.1
Fat (g)	14.2	9.9	21.4	20.9
Carbohydrate (g)	48.6	70.5	52.0	66.7

ICS: Instant cream soup; IPD: Instant powdered-drink; SB: Snackbar; GSF: Government supplementary food

The products' consumption was monitored using a compliance form to record daily products consumption. The compliance form, used package, and product-waste should be returned once a week. After eight weeks, endline data collection was performed.

Data analysis

The collected data was processed and analyzed by using Microsoft Excel 2013 and SPSS Version 16.0. Maternal characteristics were analyzed descriptively. Independent t-test or Mann-Whitney test was performed to analyze the difference between the two groups. Paired T-test was performed to analyze the difference of fetal growth after intervention. Adjusted ANCOVA analyses was performed to determine the effect of intervention on fetal growth by adjusting the covariate variables.

RESULTS AND DISCUSSION

Maternal characteristics

Maternal characteristics, namely maternal age, age of gestation, gap between pregnancy, parity, history of stillbirth, pre-pregnancy body mass index (BMI), and MUAC were examined and none showed any significant difference (Table 2).

Most subjects in the PG and PCG were aged between 17–25 years with a mean maternal aged of 25.92±4.4 years and 23.95±3.6 years, respectively. The maternal age range with the lowest risk is 20–30 years (Bellieni 2016). Teen pregnancies increases the risk of pre-term labor, Low Birth Weight (LBW), post-partum hemorrhage, and maternal or infant mortality (Kemenkes RI 2015). The gestation age in both groups were ranged between 19–21 weeks. During this period, accelerated fetal growth, structural development, and the beginning of functional activity start to occur (Wiknjosastro *et al*. 1992).

Most of the subjects had a gap between pregnancy of more than two years, this in line to the WHO recommendation (2006). Almost all of the subjects belonged to the nullipara and primipara. Mothers who have had >5 parity have higher risks of fetal macrosomia, diabetes mellitus, and pregnancy induced hypertension (Alsammani & Ahmed 2015). A few of subjects had experienced miscarriage. Teen pregnancies,

as well as advanced maternal age, and maternal malnutrition are the risk factors of miscarriage (Metwally *et al*. 2010). Mean maternal pre-pregnancy BMI in the PG and PCG groups were 18.82±2.2 kg/m2 dan 18.7±2.1 kg/m2, respectively. Despite all mothers involved in the study had a MUAC of below 23.5 cm, only around half were categorized as underweight based on their BMI in both groups. According to Fakier *et al*. (2017) MUAC can significantly define maternal nutritional status up to 30 weeks of pregnancy.

Maternal nutrient intake (other than supplemented foods)

Mean nutrients intake were obtained using the 24 hours food recalls which were conducted four times through intervention period. All mean nutrients intake of subjects in both groups were below the dietary recommendation intake for pregnant women (AKG 2013) the subjects nutrient adequacy level was below 70%. The mean intake of all nutrients in both groups did not differ significantly (Table 3).

The mean energy intake in both groups were below the daily recommendation intake for pregnant women, which was 2,550 kkal per day. The subjects' mean energy intake in both groups were approximately 1,600 kkal. The level of energy intake during the gestational period correlated significantly with the infant birth weight. Maternal energy intake of below 1,500 kkal will result in infant LBW (Durrani & Rani 2011). The subjects' low energy intake was caused by low frequency in food consumption, which was less than 3 times a day. Low frequent food intake during pregnancy increases the risk of maternal CED (Engidaw *et al*. 2019).

The mean protein intake of the subjects in both groups were approximately 55 g per day, while the recommendation of protein intake for pregnant women is 76 g per day. According

Table 2. Maternal characteristics in intervention group and positive control group

Indicators	Group		p value
	PG (n=24)	PCG (n=20)	
Maternal age (years)	25.92±4.4	23.95±3.6	0.118
<17 years (n,%)	1 (4.2)	0 (0.0)	
17-25 years (n,%)	13 (54.2)	14 (70.0)	
>25 years (n,%)	10 (41.6)	6 (30.0)	
Gestation age (weeks)	19.7±4.1	20.9±3.8	0.313
Pregnancy gap[2]			0.408
<24 months (n,%)	5 (20.8)	3 (15.0)	
>24 months (n,%)	10 (41.6)	7 (35.0)	
1st pregnancy (n,%)	9 (37.6)	10 (50.0)	
Parity[2]			0.550
Nullipara (n,%)	10 (41.6)	9 (45.0)	
Primipara (n,%)	10 (41.6)	10 (50.0)	
Multipara (n,%)	4 (16.7)	1 (5.0)	
Miscarriage[2]			0.526
Yes (n,%)	4 (16.7)	2 (10.0)	
No (n,%)	20 (83.3)	18 (90.0)	
Pre-pregnancy BMI (kg/m^2)	18.82±2.2	18.7±2.1	0.865
Underweight (n, %)	11 (45.8)	11 (55.0)	
Normal (n, %)	13 (54.2)	9 (45.0)	
MUAC (cm)	21.35±1.2	21.28±1.3	0.848

Independent T-test: [2]Mann-Whitney; Significant at p<0.05; PG: Intervention Group; PCG: Positive Control Group; MUAC: Mid Upper Arm Circumference

to Kominiarek & Rajan (2016), the minimum amount of daily protein intake to assure optimal fetal growth during pregnancy is 60 g per day. The low protein intake happened because of low intake of protein-sourceds foods consumption as obtained through FFQ. This low protein intake can lead to impaired fetal growth and development, cretinism, and IUGR (intrauterine growth restriction) (Wu *et al*. 2012).

According to Kemenkes RI (2014), the total fat intake should comprise 20–30% of the daily calories intake. The mean of maternal fat adequacy level for both groups were higher than the recommendation. On the other hand, the total carbohydrate intake should comprise 45–65% of daily calories intake (Kemenkes RI 2014). The mean maternal carbohydrate adequacy level of both groups were considered to be sufficient. This condition showed that all of the subjects had low energy intake, with fat and carbohydrate as the major macronutrient sources for their daily calories intake.

The recommended type of fatty acid during pregnancy are docosahexaenoic acid and eicosapentaenoic acid, which are beneficial for fetal brain development and proper functioning of the retina (Danielewicz *et al*. 2017). The major fat source of subjects were saturated fatty acid because most of the subjects consumed fried food in their daily food intake. Frequent high saturated fats consumption during pregnancy causes maternal and infant implications, such as anemia, impaired fetal growth and development, and LBW and/or excess weight (Santana *et al*. 2015). A study conducted using animal model showed that maternal high saturated fats consumption increased the plasma total cholesterol and low density lipoprotein cholesterol concentrations in the offsprings (Chechi & Cheema 2006).

Complex carbohydrate is recommended for pregnant women to improve the function of gut microbiome (Danielewicz *et al*. 2017). Maternal carbohydrate source in this study were mostly simple carbohydrate in the form of white rice or flour-based food. According to Starling *et al*. (2017), pregnant women with high intake of eggs, starchy vegetables, and nonwhole grains was correlated with higher maternal fasting glucose and greater newborn adiposity, than the pregnant women with high intake of whole grains, poultry, nuts, and cheese.

Compliance level of supplementary foods consumption

Maternal compliance level in PG was lower than PCG and the difference was statistically significant (Table 4). The low compliance level in PG group happened because there were three variances of products with diverse tastes and serving methods. Meanwhile the GSF which were given to subjects in PCG was more efficient and easy to be consumed, so the compliance level was higher.

The compliance level of each products in the PG were described in Figure 1. Low compliance level on ICS and IPD consumption might happened because of subjects' infamiliarity to consume those products as supplementary foods, especially ICS which was uncommon to be consumed in Indonesia. Cream soup should be consumed as an appetizer or main course (Setiawati *et al*. 2017). The subjects could not consumed the entire ICS since it was way too fulfilling if consumed as supplementary food.

Table 3. Mean nutrient intake and adequacy level of subjects in intervention group and positive control group

Nutrients	Intake and adequacy level	Group		p-value
		PG	PCG	
Energy	Intake (kkal)	1,619±480	1,687±436	0.631
	Adequacy (%)	63.5±18.8	66.1±17.1	0.629
Protein[2]	Intake (g)	56.3±17.7	55.5±13.8	0.962
	Adequacy (%)	74.1±23.3	73.0±18.2	0.953
Fat	Intake (g)	55.0±18.2	61.7±19.4	0.241
	Adequacy (%)	30.6±4.1	32.8±4.2	0.240
Carbohydrate[2]	Intake (g)	224.6±70.0	234.4±64.8	0.580
	Adequacy (%)	55.4±4.5	55.5±3.4	0.580

Independent T-test: [2]Mann-Whitney; Significant at $p<0.05$; PG: Intervention group; PCG: Positive control group

IPD should be dissolved by hot water. However, although it had been already dissolved by hot water, it still had thick texture and not-entirely dissolved, so it contained residual which stayed in the mouth and left certain after taste after being swallowed. Similar with the IPD, the ICS had the same problems and resulted in low compliance level. Moreover, ICS was uncommon for the subjects, so majority of the subjects disliked the product. The highest compliance of protein sourced supplementary foods was from the SB, since the product was more efficient to be served and the taste was also more preferable than the others. Figure 1 Compliance level of each supplemented foods in PG.

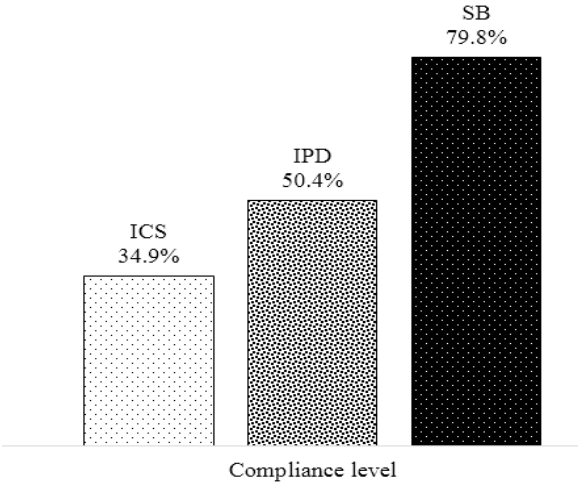

Figure 1. Compliance level of each supplemented foods in PG

Effect of protein-sourced supplementary foods on fetal growth

Fetal growth was determined by using two indicators, femur length (FL) and estimated fetal weight (EFW). Table 5 indicated the average growth of fetuses in each gestation week. Low birthweight may happened due to intrauterine growth restriction, preterm delivery, or both. Birthweight is also a determinant risk of noncommunicable diseases occurence, with cardiovascular disease, type II diabetes mellitus, and obesity as the prominent evidences. Fetal growth observation should be done during pregnancy period as an early detection of imbalanced fetal growth. According to Kiserud *et al*. (2017) the fetal growth in both groups were already in accordance with the current gestation week, however there was no difference in both measurement of indicators in both groups.

The FL and EFW in both groups had improved significantly at the endline data collection (Table 6). However, the difference in both groups were not significant. Thus the PG had a similar effect with GSF in the PCG on fetal growth regardless of its lower compliance. After the covariate variables were adjusted using ANCOVA analysis, the result also showed no significant effect of the two supplementary foods on fetal growth.

Table 4 Subjects distribution according to level of compliance

Compliance	Group		p value
	PG (n=24)	PCG (n=20)	
	65.0±9.1	85.3±2.6	0.000*
High (≥70%)	7 (30.4)	17 (85.0)	
Moderate (50-70%)	14 (56.6)	1 (5.0)	
Low (<50%)	3 (17.0)	2 (10.0)	

Independent T-test: Significant at p<0.05; PG: Intervention group; PCG: Positive control group

The small impact of the supplementary food on fetal growth may occur because of the short intervention period and low compliance to the supplements. This result was similar to Nahar *et al*. (2009) who reported that small impact of supplementary food on maternal weight gain, and the difference could be explained by the time of supplementation starts, compliance, scheduled monitoring and supervision, also the nutrient content of the supplements. The compliance level in PG was significantly lower than the PCG and resulted on the products' nutrient contents were partially consumed. However, it can be assumed that if the protein-sourced supplementary foods has a better compliance, it will give better result than the GSF. A better compliance of supplemented food showed an increase in infant mean birth weight up to 180 g (Tabrizi *et al*. 2019). Malnourished and anemic women tended to have higher proportions on LBW (Mahajan *et al*. 2009), However, intervention to improve their nutritional status such as administration of supplementary food showed more positive effects on lowering LBW and preterm births in this group (Tabrizi *et al*. 2019).

Based on the adjusted ANCOVA analysis, the covariate factors which were affecting the fetal growth were the maternal height for FL ($p<0.05$) and the gestational age, both for FL and

Table 5. Average growth of fetuses in each gestation week in both groups

Group	Fetal measurement	Gestation week			
		21–25	26–30	31–35	36–38
PG	Estimated fetal weight (g)	567.5±194.4	1,290,7±300.0	222.8±349.9	2,540±56.6
	Femur length (mm)	41.1±3.6	54.4±3.5	64.7±3.7	70.9±0
	Total	4	6	12	2
PCG	Estimated fetal weight (g)	388	1,187±302.7	1,956,4±330.1	2,974±185.3
	Femur length (mm)	32.9	52.9±4.6	62.6±3.6	73.9±1.6
	Total	1	5	12	2

PG: Intervention group; PCG: Positive control group

EFW ($p<0.05$). This study was started when the subjects were in the 2nd trimester of pregnancy and ended in the 3rd trimester of pregnancy. Maternal height gave the information on genetic potentials of the offsprings. Fetal FL was found to be positively related with maternal height on the last stage of pregnancy (29 weeks to birth) (Wills *et al*. 2010). Another study also showed similar effect and stated that positive relation between maternal height and fetal FL on singleton pregnancy at 25 weeks of pregnancy (Goldenberg *et al*. 1993).

Both of the fetal growth indicators in this study were positively correlated with gestational age. Women experienced hormone secretion changes during pregnancy which resulted on the alteration of carbohydrate, fat, and protein function. Adaptive responses were developed during pregnancy to ensure sufficient nutrient for fetus, regardless of the maternal nutritional status (Williamson 2006). Fetal growth increase rapidly at the last trimester of pregnancy, marked by the rapid growth and improvement of brain and organs functions (Santrock 2012). However, poor outcomes might still be present both for mothers and the offsprings, such as increase of maternal and infant mortality, preterm delivery, and infant LBW, if the maternal nutritional needs still not be fulfilled (Erick 2008; Medhin *et al*. 2010).

Table 6. Effect of variation of protein-sourced supplementary foods on fetal growth

Growth	Group		p-value
	PG	PCG	
Femur length (mm)			
Baseline	31.28±12.2	33.97±12.4	
Endline	58.70±10.0	59.8±9.4	
p-value[1]	0.000*	0.000*	
Δ[2]	27.42±5.0	25.85±6.1	0.357
Adjusted Ancova[3]	(26.692)	(26.722)	0.982
Estimated fetal weight (g)			
Baseline	408.08±297.46	474.65±270.89	
Endline	1,740,29±743.09	1,787,4±669.6	
p-value[1]	0.000*	0.000*	
Δ[2]	1,332,21±515. 9	1,312,75±487.0	0.899
Adjusted Ancova[3]	(1,250)	(1,411)	0.293

[1]Paired T-test: Significant at $p<0.05$

[2]Independent T-test: Significant at $p<0.05$

[3]ANCOVA: Significant at $p<0.05$; Covariate: Maternal age, maternal height, maternal pre-pregnancy BMI, gestational age gain, maternal weight gain, parity

PG: Intervention Group; PCG: Positive control group

CONCLUSION

The protein-sourced supplementary foods had lower compliance level than GSF, however both intervention showed similar effects on fetal growth (adjusted ANCOVA) ($p>0.05$). The main factors that affected the fetal growth were gestational age and maternal height. Optimal nutritional intake and supplementary food consumption based on the recommendations for CED pregnant women can improve fetal growth. Improvement on the organoleptic aspects of the current product may lead to increase in the compliance level, hence it may show better result on fetal growth. In addition, longer intervention period and addition of micronutrients to the product may also support improvement on fetal growth indicators.

ACKNOWLEDGEMENT

This research was supported by Kementerian Kesehatan Republik Indonesia (Kemenkes RI). We thank the health workers in Puskesmas Gang Kelor, Puskesmas Semplak, Puskesmas Merdeka, Puskesmas Sindang Barang, Puskesmas Pasir Mulya, Puskesmas Pancasan, Puskesmas Kayu Manis, Puskesmas Tanah Sareal, and Puskesmas Pondok Rumput Kota Bogor. Also the cadres in the involved ante-natal care services. The authors have no conflict of interest.

REFERENCES

Alsammani MA, Ahmed SR. 2015. Grand multiparity: Risk factors and outcome in a tertiary hospital: A comparative study. Mater Sociomed. 27(4): 244–247. DOI: 10.5455/msm.2015.27.244-247.

Bellieni C. 2016. The best age for pregnancy and undue pressures. J Fam Reprod Health. 10(3):104–107.

Black ER, Heidkamp R. 2018. Causes of stunting and preventive dietary interventions in pregnancy and early childhood. Nestlé Nutr Inst Workshop Ser 89:105–113. https://doi.org/10.1159/000486496.

Brown JE 2005. Nutrition through the Life Cycle 2nd Ed. Belmont: Thomson Wadsworth.

Chechi K, Cheema SK. 2006. Maternal diet rich in saturated fats deleterious effects on plasma lipids of mice. Exp Clin Cardiol. 11(2):129–135.

Danielewicz H, Myszczyszyn G, Dębińska A, Myszkal A, Boznański A, Hirnle L. 2017. Diet in pregnancy-more than food. Eur J Pediatr 176:1573–1579. https://doi.org/10.1007/s00431-017-3026-5.

Dewey KG. 2016. Reducing stunting by improving maternal, infant, and young child nutrition in regions such as South Asia: Evidence, challenges and opprtunities. Matern Child Nutr 12:27–38. DOI: 10.1111/mcn.12282

Durrani AM, Rani A. 2011. Effect of maternal dietary intake on the weight of the newborn in Aligarh city, India. Niger Med J. 52(3):177–181.doi:10.4103/ 0300-1652.86132.

Engidaw MT, Gebremariam AD, Tiruneh SA, Asnakew DT, Abate BA. 2019. Chronic energy deficiency and its associated factors among lactating women in Debre Tabor general hospital, Northcentral Ethiopia. Journal of Family Medicine and Health Care 5(1):1–7.doi:10.11648/j.jfmhc.20190501.11.

Erick M. 2008. Nutrition during pregnancy and lactation. In: Mahan LK, Stump SE, editor. Krause's Food and Nutrition Theraphy 12th Ed. Vancouver: Saunders Elsevier Inc.

Fakier A, Petro G, Fawcus S. 2017. Mid-upper arm circumference: A surrogate for body mass index in pregnant women. S Afr Med J 107(7):606–610. https://doi.org/10.7196/SAMJ.2017.v107i7.12255.

Goldenberg RL, Davis RO, Cliver SP, Cutter GR, Hoffman HJ, Dubard MB. 1993. Maternal risk factors and their influence on fetal anthropometric measurements. Am J Obstet Gynecol 168(4):1197–1205. https://doi.org/10. 1016/0002-9378(93)90369-t.

Imdad A, Bhutta ZA. 2012. Maternal nutrition and birth outcomes: Effect of balanced protein-energy supplementation. Paediatr Perinat Epidemiol 26(1):178–190. https://doi.org/10.1111/j.1365-3016.2012.01308.x.

[Kemenkes RI] Kementerian Kesehatan Republik Indonesia. 2007. Riset Kesehatan Dasar. Jakarta: Kemenkes RI.

[Kemenkes RI] Kementerian Kesehatan Republik Indonesia. 2018a. Riset Kesehatan Dasar. Jakarta: Kemenkes RI

[Kemenkes RI] Kementerian Kesehatan Republik Indonesia. 2018b. Kementerian Kesehatan Republik Indonesia. Buku Saku Pemantauan Status Gizi Tahun 2017. Jakarta: Kemenkes RI.

[Kemenkes RI] Kementerian Kesehatan Republik Indonesia. 2013. Riset Kesehatan Dasar. Jakarta: Kemenkes RI.

[Kemenkes RI] Kementerian Kesehatan Republik Indonesia. 2014. Pedoman Gizi Seimbang. Jakarta: Kemenkes RI.

[Kemenkes RI] Kementerian Kesehatan Republik Indonesia. 2015. Infodatin: situasi kesehatan reproduksi remaja. http://www.depkes.go.id/article/view/15090200001/situasi-kesehatan-reproduksi-remaja.html. [Accessed 19 May 2015].

[Kemenkes RI] Kementerian Kesehatan Republik Indonesia. 2017. Petunjuk Teknis Pemberian Makanan Tambahan (Balita–Ibu Hamil–Anak Sekolah). Jakarta: Kemenkes RI.

Kaseb F, Kimiagar M, Ghafarpoor M, Valaii N. 2002. Effect of traditional food supplementation during pregnancy on maternal weight gain and birthweight. Int J Vitam Nutr Res 72(6):389–393. https://doi.org/10.1024/0300-9831.72.6.389.

Kiserud T, Piaggio G, Carroli G, Widmer M, Carvalho J, Jensen LN, Giodano D, Cecatti JG, Aleem HA, Talegawkar SA *et al*. 2017. The world health organization fetal growth chart: A multinational longitudinal study of ultrasound biometric measurements and estimated fetal weight. PLoS Med 14(1):1–36. https://doi.org/10.1371/journal.pmed.1002220.

Kominiarek MA, Rajan P. 2016. Nutrition recommendations in pregnancy and lactation. Med Clin N Am 100(6):1199–1215. https://doi.org/10.1016/j.mcna.2016.06.004.

Liberato SC, Singh G, Mulholland K. 2013. Effects of protein energy supplementation during pregnancy on fetal growth: A review of the literature focusing on contextual factors. Food Nutr Res 57(1):20499. https://doi.org/10.3402/fnr.v57i0.20499

Mahajan SD, Singh S, Shah P, Gupta N, Kochupillai N. 2009. Effect of maternal malnutrittion and anemia on endocrine regulation of fetal growth. Endocrine Research 30(2):189–203. https://doi.org/10.1081/ERC-200027380.

Medhin G, Hanlon C, Dewey M, Alem A, Tesfaye F, Worku B, Tomlinson M, Prince M. 2010. Prevalence and predictors of undernutrition among infants aged six and twelve months in Butajira, Ethiopia: The P-Mamie birth cohort. BMC Public Health 10(27):1–15. https://doi.org/10.1186/1471-2458-10-27.

Metwally M, Saravelos SH, Ledger WL, Li TC. 2010. Body mass index and risk of miscarriage in women with recurrent miscarriage. Fertil Steril 94(1):290–295. https://doi.org/10.1016/j.fertnstert.2009.03.021.

Nahar S, Mascie-Taylor CN, Begum HA. 2009. Impact of targeted food supplementation on pregnancy weight gain and birth weight in rural Bangladesh: An assessment of the Bangladesh Integrated Nutrition Project (BINP). Publ Health Nutr 12(08):1205–1212. https://doi.org/10.1017/S1368980008003765.

Sairuroh, Rahfiludin MZ, Kartasurya MI . 2019. Dampak pemberian biskuit pada ibu hamil berisiko kekurangan energi kronis terhadap kadar hemoglobin. MKMI 18(2):1–6.

Santana JDM, Queiroz VADO, Brito SM, Santos DBD, Assis AMO. 2015. Food consumption patterns during pregnancy: a longitudinal study in a region of the North East of Brazil. Nutr Hosp 32(1):130–138. http://dx.doi.org/10.3305/nh.2015.32.1.8970.

Santrock JW. 2012. Life-span Development 13th Ed: Perkembangan Masa Hidup Jilid 1. Jakarta: Erlangga.

Setiawati T, Sudewi, Mahmudatussa'adah A. 2017. Sweet potato cream soup sebagai alternatif bisnis makanan sehat. Jurnal Kompeten Teknik 9(1):1–6. https://doi.org/10.15294/jkomtek.v9i1.12184.

Starling AP, Sauder KA, Kaar JL, Shapiro ALB, Siega-Riz AM, Dabelea D. 2017. Maternal dietary patterns during pregnancy are associated with newborn body composition. J Nutr 147:1334–1339. https://doi.org/10.3945/jn.117.248948.

Tabrizi JS, Asghari A, Pourali F, Kousha H, Nikniaz L. 2019. Effects of food supplementation during pregnancy on maternal weight gain, hemoglobin levels

and pregnancy outcomes in Iran. Matern Child Health J 23(2): 258–264. https://doi.org/10.1007/s10995-018-2648-1.

[TNP2K] Tim Nasional Percepatan Penanggulangan Kemiskinan. 2017. 100 Kabupaten/Kota Prioritas untuk Intervensi Anak Kerdil (Stunting). Jakarta: Sekretariat Wakil Presiden RI.

[WHO] World Health Organization. 1995. Maternal Anthropometry and Pregnancy Outcomes: A WHO Collaborative Study. Geneva: World Health Organization.

[WHO] World Health Organization. 2006. HTSP 101: everything you want to know about healthy timing and spacing of pregnancy. http://www.who.int/pmnch/topics/maternal/ htsp101.pdf. [Accressed 19 May 2019].

[WHO] World Health Organization. 2011. WHO Recommendations for Prevention and Treatment of Pre-eclampsia and Eclampsia. Geneva: World Health Organization.

[WHO] World Health Organization. 2013. Diagnostic Criteria and Classification of Hyperglycemia First Detected in Pregnancy. Geneva: World Health Organization.

[WHO] World Health Organization. 2017. Balanced energy and protein supplementation during pregnancy. eLENA. http://www. who.int/elena/titles/energy_protein_pregnan cy/en/. [Accessed 16 October 2018].

Wiknjosastro H, Saifuddin AB, Rachimhadhi T. 1992. Ilmu Kebidanan. Jakarta: Yayasan Bina Pustaka Sarwono Prawirohardjo.

Williamson CS. 2006. Nutrition in pregnancy. Nutr Bull 31(1):28–59. https://doi.org/10.1111/j.1467-3010.2006.00541.x.

Wills AK, Chinchwadkar MC, Joglekar CV, Natekar AS, Yajnik CS, Fall CHD, Kinare AS. 2010. Maternal and paternal height and BMI and patterns of fetal growth: The pune maternal nutrition study. Early Hum Dev 86(9):535–540. https://doi.org/10.1016/j.earlhumdev.2010.07.002.

Wu G, Imhoff-Kunsch B, Girard AW. 2012. Biological mechanisms for nutritional regulation of maternal health and fetal development. Paediatr Paediatr Perinat Epidemiol 26(1):4–26. https://doi.org/10.1111/j.1365-3016.2012 .01291.x.

Available online: http://journal.ipb.ac.id/index.php/jgizipangan ***J. Gizi Pangan, March 2020, 15(1):11-18***
Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019 *DOI: 10.25182/jgp.2020.15.1.11-18*
ISSN 1978-1059 EISSN 2407-0920

Effect of Biscuit Enriched with Bilih Fish (*Mystacoleucus padangensis*) on Growth of Experimental Rats

Fivi Melva Diana[1*], Rimbawan[2], Evy Damayanthi[2], Mira Dewi[2], Vetnizah Juniantito[3], Nur Indrawaty Lipoeto[4]

[1]Faculty of Public Health, Andalas University, Padang 25137, Indonesia
[2]Department of Community Nutrition, Faculty of Human Ecology, IPB University, Bogor 16680, Indonesia
[3]Faculty of Veterinary Medicine, IPB University, Bogor 16680, Indonesia
[4]Medical Faculty, Andalas University, Padang 25128, Indonesia

ABSTRACT

The research aimed to produce biscuits formulated with Bilih fish and to analyse the effect of Bilih-fish-based biscuits on the growth of experimental rats. Six biscuit formulas were developed using three different amounts of Bilih fish flour (0 g, 20 g, and 30 g added to one formula dough) and two flavors (chocolate and vanilla). One biscuit formula was selected based on the best organoleptic test. Four treatments were applied to 24 male growing rats (21 day olds of the Spraque Dawley strain) for 28 days using a completely randomized design study. The treatments used were P1 (standard feed), P2 (standard feed + standard biscuits), P3 (standard feed + standard biscuits + pure omega-3 oil) and P4 (standard feed + the best formula biscuit). The parameters measured on rats were weight and tail length. The best formula biscuit based on the organoleptic test was the chocolate-flavor biscuits prepared with 20 g Bilih fish flour. The longest tail length was found in the P4 treatment (2,257±0.52 cm), which was significantly longer than the P2 (1.46±0.34 cm) and P1 (1.34±0.29 cm) treatments. No significant weight differences were found among the rats in all treatments. Hence, the chocolate-flavor biscuits formulated with the addition of 20 g Bilih fish flour increased the linear growth in experimental rats as shown by the significant difference in the tail lengths found in the treatment group.

Keywords: bilih fish flour, biscuit formulation, omega-3, tail length, weight

INTRODUCTION

The first 1,000 days of life is a golden period for child development. It starts with 270 days during pregnancy, followed by 730 days or two years after birth (BAPPENAS 2015). Omega-3 fatty acids play an important role in the growth and development within the first 1,000 days. A previous study has reported that the lack of omega-3 fatty acids consumption may cause stunting in humans (Diana 2013).

Many studies on experimental animals also show that omega-3-fatty-acid-deficient diet is associated with faltered growth. One of the omega-3 fatty acid components that plays an active role in growth is the DHA (docosahexaenoic acid). In addition, other studies have also mentioned that omega-3 fatty acids in the diet play an important role in fetal growth. However, the clinical mechanisms on how the omega-3 fatty acids affect growth still needs further research (Bernardi 2012).

Omega-3 fatty acids and proteins are widely present in various types of fish, and the increased consumption of local fish will improve the nutritional status in children and the local economy. However, fish has certain organoleptic properties which are not necessarily favored by children. Thus, there is a need to develop fish-based products that are able to improve acceptance among children as consumers.

Widodo (2015) had demonstrated the use of locally processed food products with freshwater fish in the form of biscuits, namely the snakehead-murrel-based biscuits that can improve the nutritional status of under-five-year-old children. Biscuits were chosen because they can be consumed by all ages and socio-economic strata, are easy to store, have a long shelf life and are easy to produce and distribute. Furthermore, they can also be adjusted to the child's digestion and be shaped in such a way as to increase the children's acceptance.

[*]**Corresponding Author:** tel: +6285263610820, email: fividiana0503@gmail.com

Bilih fish (*Mystacoleucus padangensis*) is a local fish from Solok, West Sumatera. It contains nutrients such as proteins, fats, vitamins, and minerals (Syandri 2008). The results of the preliminary study in this research show that Bilih fish contains a high level of omega-3 fatty acids (0.553 g/100 g of fresh fish). Thus, it is important to study the potential Bilih fish has as an ingredient for nutritious biscuits development to improve children growth. The study should start with an animal study before further steps in human subject are taken. The study offers scientific evidence on the development process and effect of biscuits enriched with Bilih fish that is rich in omega-3 fatty acids in an animal study, which serves a basis for a further human study.

METHODS

Design, location, and time

The experimental design used was the Completely Randomized Design (CRD). The biscuit development was conducted in the Laboratory of the Department of Community Nutrition, IPB University, whereas macro- and micronutrient analyses were conducted at the GIS Laboratory (Saraswanti Indo Genetech), Bogor, West Java. Meanwhile, the growth assessment of the research animal was conducted at the Animal Veterinary Hospital (RSHP), Faculty of Veterinary Medicine, IPB University. This research was conducted from March 2016 until January 2018. The study had obtained an ethical clearance number 72–2017 from the Animal Ethics Committee of the Institute of Research and Community Service, IPB.

Materials and tools

The material used in this research was Bilih fish that was made into flour and then formulated into biscuits. The biscuits were made from wheat flour, egg, fine sugar, margarine, full cream milk, vanilla powder, and chocolate powder. The fish flour was made with dump dryer, oven, blender, grinding machine, spoon, spatula, knife, basin, pan, wok, blender, mixer, biscuit mold, flour sieve, and stove. The animal subjects were white rats aged 21 days of the Sprague Dawley strain.

Procedure

The research was divided into two phases. The first phase was the development of the Bilih Fish-based biscuits, and the second phase was the study of the effect of biscuit intervention on the rats' growth.

Bilih fish-based biscuit development. The Bilih fish used were around 4.0–8.5 cm in length and 0.5–5.1 g in weight. The fish was caught at 100–300 yards depth of Singkarak Lake at relatively low water temperatures between 24.0–26.0°C.

Fish flour preparation. The process of making the fish flour began by cleaning the fish then drying it under the sun for approximately 11 hours/day from 7 am until 6 pm over 7 days, making up a total of 77 hours, until the moisture content reached ±4%. The dried fish was then smoothed in a drum dryer at a temperature of 80°C for approximately 15 minutes for 1 kg of fresh Bilih fish, then mashed and sieved with a 60-mesh sieve. The fish flour obtained from 1 kg of fresh fish was around 290–320 g, or in the ratio of about 1:3, with light brown color and a smooth texture. The fish flour was then analysed for nutrient content before being used as an ingredient in biscuits.

Formulation of the biscuit product made from Bilih fish. The aim of this phase was to develop the biscuit using six biscuit formulas. Table 1 shows the dough formulation of Bilih fish biscuits, then the biscuits were molded and baked for 35 minutes at temperatures of 160–165ºC, and then cooled.

The amount of full cream milk used in the chocolate-flavored biscuit formula was lower than that used in the vanilla-flavored biscuit formula because some of the weight of the full cream milk was replaced by chocolate powder to result in the same weight of dry inggridients. The organoleptic test used was the hedonic test that involved 40 respondents. Respondents assessed the color, flavor, taste, texture, and overall quality of the six biscuit product formulas (Table 1). The selection of the best biscuit formula was done based on the respondents' hedonic test, in which the biscuits were assessed and assigned a score of 1 to 7, detailed as follows: score 1 was interpreted as extreme dislike, 2 as moderate dislike, 3 as slight dislike, 5 as slight like, 6 as moderate like, and 7 as extreme like. For the assessment of hedonic quality test, the best formula was assessed based on the color from score 1=dark chocolate to score 7=golden yellow, based on the texture from score 1=very hard to score

Table 1. Formulation of fish-based biscuit product

Ingredients	Chocolate			Vanilla		
	F0	F20	F30	F0	F20	F30
Fish flour (g)	-	20	30	-	20	30
Wheat flour (g)	225	225	225	225	225	225
Egg yolk (unit)	1	1	1	1	1	1
Fine sugar (g	150	150	150	150	150	150
Margarine (g)	300	300	300	300	300	300
Full cream milk (g)	30	30	30	80	80	80
Vanilla powder (tsp)	0.5	0.5	0.5	0.5	0.5	0.5
Chocolate powder (g)	50	50	50	-	-	-
Liquid vanilla (tbsp)	-	-	-	2	2	2

7=very crunchy, based on the aroma from score 1=very hard to score 7=very weak, and based on other properties from score 1=very weak to score 7=very strong (Setyaningsih *et al.* 2010). The best formula obtained from the organoleptic test was then analysed for the nutrient content. The decision making on the selection of the best formula was based on the attributes that gave the highest value after standard biscuit.

Nutrient analysis. The proximate nutrient analysis and fatty acid analysis of the fresh Bilih fish, Bilih fish flour, the biscuit with the best organoleptic properties, and the standard biscuit were performed using AOAC methods (AOAC 2005). The analyses included the protein content (Kjeldahl method), fat content (Soxhlet method), ash content (Gravimetric method), moisture content (oven method), carbohydrate content (by difference method), and total energy (by calculation).

The fatty acid content, especially the omega-3 fatty acids, in fresh Bilih fish, Bilih fish flour, the selected biscuit, and standar biscuit was analysed using the Gas Chromatography method. The procedure stages for the analyses were as follows: 1. standard solution preparation; 2. sample preparation (including a. fat content determination, b. fat extraction by cold extraction, c. methylation of fat); and 3. omega-3 fatty acids analysis with GC FID (Gas Cromatography-Flame Ionization Detector) (AOAC 2001). The chromatography conditions were as follows: column=Supelco SPTM 2560 100 m 0.25 mm 0.2 um; water rate=18.0 cm/sec with column length 100 m; carrier gas=N_2; detector FID=240ºC; injector temperature=225ºC; Split=1:100 (Khan *et al*. 2017).

The effects of biscuit intervention on the growth of Sprague dawley rats. The aim of this phase was to study the effect of administration of selected Bilih fish biscuit formula on the growth of the experimental rats. The rats were placed in pairs in a cage and in a room with a light and dark period of 12 hours each. The cage temperature ranged from 23.9ºC to 26.6ºC with an average temperature of 25.5ºC, and the air humidity ranged from 67% to 88% with an average humidity of 81%.

The rats were given standard daily rations (including water) ad libitum for 14 days of acclimatization period. All rats then entered the treatment period of 28 days. The rats were divided into four groups and were given 20 g/day of standard feed orally. In addition to that, during the treatment period each group of rats was also given one treatment feed by gastric tube feeding method. These included P1 (0.2 g standard feed + 6 ml aqua bidest per day), P2 (2.43 g of standard biscuit + 6 ml aqua bidest per day), P3 (2.43 g of standard biscuits + 0.014 g of pure omega-3 oil + 6 ml aqua bidest per day), and P4 (2.09 g of the selected formula of Bilih fish biscuits + 6 ml aqua bidest per day). The doses of pure omega-3 oil and omega-3 in the biscuits were calculated based on the National Recommended Daily Allowance for omega-3 (0.7 g/day) for children aged 1–3

years (Kemenkes 2013). Based on the Food and Drugs Administration (FDA 2005) guideline, the doses were then converted to rat doses, which were equivalent to 2.43 g/day of standard biscuit, 0.014 g/day of pure omega-3 oil, and 2.09 g/day of selected Bilih fish biscuit respectively.

During the treatment period, the rat weight and tail length were measured once every 7 days. The weight was measured using a digital weighing instrument with 0.1 g accuracy. Then, the tail length was measured using a caliper with 1 mm accuracy. The tail length was measured from the position of anus to the tip of the tail, the hair at the end of the tail excluded (Sholichah 2007).

Data analysis

Descriptive analysis was applied for the organoleptic test scores, including the average, variance, and standard deviation of any combination of the Bilih fish-based biscuits. The hedonic test scores were analysed using the Kruskal Wallis test and the post hoc Dunn test. Data on the body weights and tail lengths of the rats were analysed using the ANOVA and the post hoc Tukey test at a 95% confidence level (α=0.05) (Mattjik & Sumertajaya 2013).

RESULTS AND DISCUSSION

Research phase one: Bilih fish-based biscuit development

Bilih fish (*Mystacoleucus padangensis*) is a lake-dwelling fish that migrates upstream to spawn. The main diet of this fish is detritus and zooplankton. It also feeds on phytoplanktons and other vegetable materials that fall into the water (Kartamihardja & Sarnita 2010). Mystacoleucus padangensis fishes spawn in the stream that empties into rivers around Singkarak River like Batang Sumpur River, Paninggahan River, and Muaro Pingai River. The females release eggs at the bottom of the river, which are fertilized by the males and sink to the bottom, then drift into the lake (Kartamihardja 2015). Bilih fish is a source of proteins, fats, vitamins, and minerals (Yuniritha *et al.* 2014).

Fresh Bilih fish contains 0.553 g omega-3 fatty acids in 100 g, while the dry Bilih fish flour contains 1.691 g omega-3 fatty acids in 100 g (Table 2). According to LIPI (1999), the chemical composition of fish flour is determined by the type of the fish.

Table 2. Omega-3 fatty acid content of Bilih fish fresh and Bilih fish flour obtained from Singkarak Lake 2016

Parameters	Bilih fresh fish	Dry fish flour
Omega-3 fatty acid (g)	0.553	1.691
Alpha linolenic acid (g)	Not analysed	0.423
DHA (g)	0.237	0.572
EPA (g)	0.207	0.732
Omega-6 fatty acid (g)	0.252	1.146
Linolenic acid (g)	Not analysed	0.600
AA (g)	0.096	0.408
Moisture (%)	76.44	Not analysed

Table 3 shows the hedonic test results of the average score of each Bilih fish biscuit formula based on the characteristics such as color, aroma, taste, texture, and overall attribute. The most preferred formula was the F20 formula which featured the administration of 20 g Bilih fish flour to one formula dough with chocolate flavor. The hedonic test scores by the respondents for the F20 formula were 4.88 for color, 4.11 for flavor, 4.21 for taste, 5.46 for texture, and 4.70 for the overall acceptance. This formula was then selected as the Bilih fish biscuit administered to the experimental rats.

The omega-3 fatty acid content in the F20 biscuit (20 g Bilih fish flour added to one formula dough with chocolate flavor) was 0.669 g/100 g biscuit while the F0 biscuit or the standard biscuit contained only 0.575 g/100 g biscuit of omega-3 fatty acid. The F20 biscuit also contained DHA (0.010 g/100 g biscuit) and EPA (0.012 g/100 g biscuit), which were not found in the F0 biscuit. The ratio of omega-3 to omega-6 fatty acids in the F20 biscuit was 1:5 (Table 4).

The Indonesian RDA of omega-3 fatty acid for children aged 1–3 years is 0.7 g (Kemenkes 2013). The data in Table 4 show that 0.7 g of omega-3 fatty acids can be obtained from around 100 g of F20 biscuit as it contains 0.669 g of omega-3 fatty acids.

Table 5 presents the chemical composition of the F0 and F20 biscuits as compared to the

Table 3. Results of hedonic test of biscuits

Formula	Attributes				
	Color	Flavor	Taste	Texture	Overall
Chocolate					
F0	6.08^{c}	5.44^{c}	5.78^{b}	5.14^{ab}	5.58^{b}
F20	4.88^{a}	4.11^{ab}	4.21^{a}	5.46^{a}	4.70^{a}
F30	5.56^{b}	3.94^{ab}	3.65^{a}	5.29^{b}	4.58^{a}
Vanilla					
F0	5.73^{bc}	5.60^{c}	5.84^{b}	5.78^{c}	5.75^{b}
F20	5.45^{b}	4.35^{b}	4.15^{a}	4.76^{a}	4.63^{a}
F30	4.70^{a}	3.71^{a}	3.86^{a}	4.99^{a}	4.34^{a}
p value*	0.000	0.000	0.000	0.000	0.000

Different letters in the same column show significant differences ($p<0.05$) with Kruskal Wallis test; Colour: 1=dark chocolate to 7=golden yellow; Texture: 1=very hard to 7=very crunchy; Aroma: 1=very hard to 7=very weak; Others: 1=very weak to 7=very strong

Indonesia Recommended Daily Allowance (RDA) for children aged 1–3 years of energy, proteins, fats, carbohydrates, omega-3, omega-6, moisture, and fiber. The F20 biscuits in this study can be considered a good source of energy and omega-3 fatty acids.

Research phase two: The study of the effect of biscuit intervention on the growth of Sprague dawley rats

Table 6 shows the mean and standard deviation of rat body weights for P1, P2, P3 and P4 at six different measurement times. The ANOVA results show there was no significant difference in the body weights of the rats, although the average difference in weight gain of the rats in P4 (61.57±9.10 g) was higher than those in P3 (53.60±20.45 g), P2 (51.60±14.55 g) and P1 (45.20±18.21 g), respectively. The results were in line with those reported by Firmansyah *et al.* (2017), who state that the intervention of catfish oil fortified by omega-3 fatty acids and vitamin E increased the body weight in rats, but not significantly.

Table 7 shows the mean and standard deviation of the tail lengths of the rats for P1,

Table 4. Analysis of fatty acids in 100 g F0 and F20 chocolate biscuits

Parameters (g)	F0 Chocolate	F20 Chocolate
Omega-3 fatty acid	0.575	0.669
Alpha linolenic acid	0.574	0.663
DHA	ND	0.010
EPA	ND	0.012
Omega-6 fatty acid	3,261	3,489
Linolenic acid	3,246	3,456
AA	0.005	0.013

ND: Not detected; F0: Standard biscuit with chocolate flavor; F20: Biscuit formulated by adding 20 g Bilih fish flour to one formula dough with chocolate flavor

Table 5. Chemical composition of biscuits as compared to RDA*

Chemical composition of biscuit (/100g)	F0 chocolate	F20 chocolate	The Indonesia (RDA*)
Energy (kcal)	528.16	518.09	1,125
Protein (g)	5.34	6.66	26
Total fats (g)	29.25	27.81	44
Carbohydrate (g)	60.28	60.29	155
Omega-3 (g)	0.58	0.69	0.7
Omega-6 (g)	3.26	3.49	7.0
Moisture (g)	2.92	3.00	-
Fibre (g)	7.32	6.40	16

F0: Standard biscuit with chocolate flavor; F20: Biscuit formulated by adding 20 g Bilih fish flour to one formula dough with chocolate flavor; RDA*: Recommended daily allowance, for children aged 1–3 years

Table 6. Measurement of the weight of the rats pre- and post-treatment

Treatment	Measurement of weight (g)			
	Pre	Post	Δ	p*
P1	109.80±13.55	155.00±20.50	45.20±18.21	-
P2	93.40±17.70	145.00±28.90	51.60±14.55	0.35
P3	93.00±11.70	146.60±26.10	53.60±20.45	-
P4	100.00±14.50	161.60±19.30	61.57± 9.10	-

*not significant with p>0.05 (ANOVA test); P1=standard feed; P2=standard feed + standard biscuit; P3=standard feed + standard biscuit + pure omega-3 oil and P4=standard feed + selected Bilih fish biscuit (administration of 20 g Bilih fish flour to one formula dough with chocolate flavor

P2, P3, and P4 in six measurements. The tail lengths, at both the start and end times, have a greater length difference in P4 (2.257±0.52 cm) compared to P3 (1.64±0.34 cm), P2 (1.46±0.34 cm), and P1 (1.34±0.29 cm), respectively. The ANOVA test results show that there was a more significant difference in the tail length growth in P4 treatment compared to other treatments. The Tukey test results show that P4 (2.257±0.52 cm) was more significantly different compared to P3 (1.64±0.34 cm), P2 (1.46±0.34 cm), and P1 (1.34±0.29 cm).

The increase in weight gain tendency (although it was not significant) and significant improvement in tail length were resulted from the administration of 20 g Bilih fish flour in the chocolate flavor biscuits or the F20. The F20 contains protein (6.66 g/100 g biscuit), omega-3 fatty acid (0.669 g/100 g biscuit), alpha-linolenic acid (0.663 g/100 g biscuit), DHA (0.01 g/100 g biscuit), and EPA (0.01 g/100 g biscuit). Omega-3 fatty acid and protein are nutrients that play a role in mammalian growth.

Both of the animal and human studies also show the same results where Watkins *et al.* (2003) and Wargo *et al.* (2005) state that the addition of omega-3 and omega-6 contained in fish oil could increase the body weight. Several previous research studies also mentioned that a higher intake of omega-3 fatty acids, especially DHA and ALA, is associated with improvement of birth weight and birth length in developing countries (Bernardi 2012).

The development of myelin in the neuroendocrine cells needs adequate intake of omega-3 fatty acids (EPA, DHA). These neuroendocrine cells produce growth hormone such as somatotropin which plays a role in the growth of weight and tail length. An increase in the intake of omega-3 fatty acids leads to increasing endocrine cells and growth hormone (Stewart *et al.* 2009). Omega-3 fatty acids which then are converted into DHA also affect growth hormone such as IGF-1 (Singh 2005). As Simarmata *et al.* (2012) state, various pre-clinical and clinical studies have already demonstrated

Table 7. Measurements of tail length of rats pre- and post-treatment

Treatment	Measurement of tail length (cm)			
	Pre	Post	Δ	p*
P1	12.74±2.30	14.08±2.30	1.34±0.29[a]	-
P2	13.22±0.60	14.68±0.70	1.46±0.34[a]	0.007
P3	12.78±0.96	14.42±1.13	1.64±0.34[a]	-
P4	12.98±0.70	15.24±0.50	2,257±0.52[b]	-

Different letters in the same column show *significant differences p<0.05 (ANOVA test); P1=standard feed; P2=standard feed + standard biscuit; P3=standard feed + standard biscuit + pure omega-3 oil and P4=standard feed + selected Bilih fish biscuit (administration of 20 g Bilih fish flour to one formula dough with chocolate flavor)

the role of n-long-chain polyunsaturated fatty acids (LCPUFAs) in growth.

CONCLUSION

In conclusion, the biscuit made from 20 g Bilih fish flour added to one formula dough with chocolate flavor was found to be the best formula to achieve the nutrition density intended and the most preferred in terms of organoleptic properties. The administration of the selected biscuit to growing rats was able to significantly improve tail length and tended to increase body weight, but not significantly, in the experimental rats. Thus, Bilih fish biscuit may be considered a local-based potential alternative food that is rich in nutrients, especially omega-3 fatty acids, to help improve growth in human subjects in the future.

ACKNOWLEDGEMENT

The authors would like to thank the Directorate of Research and Community Service of the Directorate General of Research and Development Strengthening of the Ministry of Research, Technology, and Higher Education through the Competitive Grant of Doctoral Research Dissertation, which has funded this research under Contract Number: 050/SP2H/LT/DRPM/2018.

We also convey our gratitude to the Faculty of Public Health of Andalas University Competitive Grant for Doctoral Research Dissertation, Contract Number 002/BPPT/SPK/PNP/FKM/Unand-2018, as well as the Departement of Community Nutrition and Faculty of Veterinary Medicine of IPB University for supporting the research. The authors have no conflict of interest.

REFERENCES

[AOAC] Association of Official Analytical Chemist. 2005. Official Method of Analysis of AOAC International 18th Edition. Maryland (Adanya ver .ini): AOAC International.

[AOAC] Association of Official Analytical Chemistry. 2001. AOAC official Method 996.06 Fat (Total, Saturated, and Unsaturated) in Food Hydrolytic Extraction Gas Chromatographic Method First Action 1996 Revised 2001. USA.

[BAPPENAS] Badan Perencanaan Pembangunan Nasional. 2015. Rencana Aksi Nasional Pangan dan Gizi Tahun 2015–2019. Jakarta: BAPPENAS.

Bernardi JR. 2012. Fetal and neonatal levels of omega-3: Effects on neurodevelopment, nutrition, and growth. The Scientific World Journal 2012: 1–8. http://dx.doi.org/10.1100/2012/202473.

Diana FM. 2013. Omega 3 dan kecerdasan anak. Jurnal Kesehatan Masyarakat 7(2):82–88. https://doi.org/10.24893/jkma.v7i2.113 org/10.1100/2012/202473.

Firmansyah H, Roosita K, Kusharto CM, Handharyani E. 2017. Pemberian minyak ikan lele (*Clarias gariepinus*) terhadap bobot badan dan perubahan histopatologi hati, ginjal, dan otak tikus srague dawley yang diberi pakan hiperkolesterolemia. J Gizi Pangan 12(2):85–92. https://doi.org/10.25182/jgp.2017.12.2.85–92.

[FDA] Food and Drugs Administration. 2005. Guidance for Industry: Estimating the Maximum Safe Starting Dose in Intial Clinical Trial for Therapeuthics in Adult Health Volunteers. Rocklville: FDA

[Kemenkes] Kementerian Kesehatan RI. 2013. Peraturan Menteri Kesehatan RI nomor 75 tahun 2017 tentang Angka Kecukupan Gizi yang Dianjurkan bagi Bangsa Indonesia. Kemenkes RI, Jakarta.

Kartamihardja ES, Sarnita, A. 2010. Populasi Ikan Bilih di Danau Toba: Keberhasilan Introduksi Ikan, Implikasi Pengelolaaan dan Prospek Masa Depan. Jakarta: Pusat Penelitian Pengelolaan Perikanan dan Konservasi Sumberdaya Ikan.

Kartamihadja ES. 2015. Potential of Culture-Based Fisheries in Indonesian Inland Waters. In: Sena SDS, Bret AI, Simon W (eds). Perspectives on culture-based fisheries developments in Asia. Bangkok: Network of Aquaculture Centres in Asia-Pacific.

Khan MU, Hassan MF, Rauf A. 2017. Determination of trans fat in selected fast food products and hydrogenated fats of India using attenuated total reflection fourier transform infrared (ATR-FTIR) spectroscopy. J Oleo Sci 66(3):251–257. https://doi.org/10.5650/jos.ess16168.

Mattjik AA, Sumertajaya IM. 2013. Perancangan Percobaan dengan Aplikasi SAS dan Mintab. Bogor: IPB Press.

Setyaningsih D, Apriyantono A, Sari MP. 2010. Analisis Sensori untuk Industri Pangan dan Agro. Bogor: IPB Press.

Simarmata N, Tiangsa S, Faranita T, Winra P. 2012. Peranan asam lemak esensial terhadap perkembangan otak dan ketajaman penglihatan. Majalah Kedokteran Nusantara 45(3):177–181.

Singh M. 2005. Essential fatty acids, DHA and human brain. Indian J Pediatr 7(3):239–242. https://doi.org/10.1007/BF02859265.

Stewart CP, Christian P, LeClerq SC, West KP, Khatry SK. 2009. Antenatal supplementation with folic acid + iron + zinc improves linear growth and reduces peripheral adiposity in school-age children in rural Nepal. Am J Clin Nutr 90(1):132–140. https://doi.org/10.3945/ajcn.2008.27368.

Sholichah Z. 2007. Mengenal Jenis Tikus. Balaba 5(2):18–19.

Syandri, H. 2008. Ancaman terhadap plasma nutfah ikan Bilih (*Mystacoleucus padangensis* Blkr) dan upaya pelestariannya di Danau Singkarak. [Scientific Oration at the Inauguration Ceremony of the Permanent Professor of the Faculty of Fisheries and Marine Sciences, Bung Hatta University]. Padang: Bung Hatta University Press.

Wargo SG, Jacobs J, Auestad N, O'Connor LO, Moore JJ, Lerner E. 2005. Body composition in preterm infants who are fed long-chain polyunsaturated fatty acids: A prospective, randomized, controlled trial. Pediatric Research 57(5):712–718.

Watkins BA, Li Y, Lippman HE, Feng S. 2003. Modulatory effect of omega-3 polyunsaturated fatty acids on osteoblast function and bone metabolism. Prostaglandins Leukotrienes and Essential Fatty Acids 68(1):387–398. https://doi.org/10.1016/S0952-3278(03)00063-2.

Widodo S. 2015. Perbaikan Status Gizi Anak Balita Dengan Intervensi Biskuit Berbasis Blondo, Ikan Gabus (*Channa striat*a), dan Beras Merah (*Oryza nivara*). [Disertation]. Bogor: IPB.

Yuniritha E, Juffrie M, Ismail D, Pramono S. 2014. The analysis of nutrient, and acute toxicity test extracts of billih fish (*Mystacoleucus-padangensis*) as local food quality for zinc supplementation. Food Science and Quality Management 33:97–104.

Available online: http://journal.ipb.ac.id/index.php/jgizipangan ***J. Gizi Pangan, March 2020, 15(1):19-26***
Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019 *DOI: 10.25182/jgp.2020.15.1.19-26*
ISSN 1978-1059 EISSN 2407-0920

Leucine Intake as Determinant of Muscle Strenght and Gait Speed in Elderly

Ratmawati[1*], Siti Fatimah-Muis[2], Muchlis Achsan Udji Sofro[3,4]
[1]Health Polytechnic Pangkalpinang, Pangkalpinang 33148, Indonesia
[2]Department of Nutrition Science, Faculty of Medicine, Diponegoro University, Semarang 50275, Indonesia
[3]Department of Internal Medicine, Faculty of Medicine, Diponegoro University, Semarang 50275, Indonesia
[4]Central Public Hospital of Dr. Kariadi, Semarang 50244, Indonesia

ABSTRACT

The purpose of this study was to analyse the intake of leucine as a determinant of muscle strength and gait speed in the elderly. This research is a cross-sectional study that involved 132 elderly people in the community (34 men and 98 women), aged 60–69 years. Data were obtained from interviews, physical examinations (body weight, body height, muscle mass, muscle strength, and gait speed), as well as food recall 3x24 hours. The statistical analysis employed Pearson's correlation coefficient and multiple linear regression. Intake of leucine was positively correlated with muscle strength ($r=0.3$; $p<0.001$) and gait speed ($r=0.244$; $p<0.05$). It was not intake of leucine that primarily determined muscle strength and gait speed but adequacy of energy intake.

Keywords: elderly, gait speed, leucine, muscle strength

INTRODUCTION

An elderly person, one aged 60 years or over, will experience a decline in muscle mass, muscle strength, and gait speed in a process known as the sarcopenia process. Sarcopenia, according to the European Working Group on Sarcopenia in the Older people (EWGSOP), is a syndrome caused by many factors resulting in decreased skeletal muscle mass, muscle strength, and physical function, with a risk of physical disability, diminished quality of life, increased risk of falls, effect on independency, as well as death (Naseeb & Volpe 2017; Deutz *et al*. 2014; Cruz-Jentoft *et al*. 2010). The Asian Working Group for Sarcopenia (AWGS) states that the prevalence of sarcopenia in Asia ranges from 2.5% to 45.7% (Wu *et al*. 2016). The study by Vitriana *et al*. (2016) shows that, based on the AWGS recommended cut-off point, the prevalence of sarcopenia in the elderly (60–85 years of age) in Bandung and Jatinangor was 9.1%, while based on the Taiwan population cut-off point, it was 40.6%. This discrepancy occurred because there was no cut-off value spesifically determined for the elderly population in Indonesia.

Skeletal muscle mass is relatively constant from a young age to adulthood, with the increase in the amount of protein absorption being offset by the increase in the amount of catabolism during fasting. Elderly people with sarcopenia experience changes in the protein turnover, which affects the balance of the protein amounts (Kim *et al*. 2010). Muscle mass, muscle strength, and gait speed are indicators for the assessment of quality of life and independence in the elderly. Protein intake, especially leucine, is important for increasing the muscle mass, muscle strength, and physical function of the elderly. The Society on Sarcopenia, Cachexia, and Wasting Disorders recommends 1.0–1.5 g of protein/kg body weight/day, including the essential amino acid leucine (Naseeb & Volpe 2017).

Essential amino acids are one of the amino acids needed by the body and can only come from food (Gropper & Smith 2013). Branched-chain amino acids (BCAA), especially leucine, help regulate the muscle protein signaling pathway (Deutz *et al*. 2014). Leucine is one of the main essential amino acids to stimulate insulin from the pancreas, protect muscles, increase energy production, accelerate healing, and repair the skin (Gropper & Smith 2013). The need for the amino acid leucine is higher (59 mg per g of protein per day) than that for other essential amino acids (WHO Report 2002). Food sources

[*]**Corresponding Author:** tel: +6281271032480 email: ummiranayumi@gmail.com

of leucine include red meat, chicken, egg white, tuna, seaweed, and soybeans (Gropper & Smith 2013).

Compared to young adults, the elderly consume fewer foods, including low energy and protein (Deutz *et al*. 2014; Kim *et al*. 2010). Based on the results of the Individual Food Consumption Survey in the Total Diet Study (Siswanto *et al*. 2014), the proportion of calorie and protein intake in elderly people aged ≥55 years nationwide was low. It was 44.6% (<70% of RDA) for calories and 45.8% (<80% of RDA) for protein. The same was found by Triatmaja *et al*. (2013) who found that the average consumption of protein in the elderly population in Panti Werdha Bandung was 32 g/day, lower than that in the average Indonesian people (55.5 g/day). Meanwhile, Kobayashi *et al*. (2013) state that total protein intake (74±14.3 g/day) was inversely correlated to the vulnerability of elderly women in Japan. Bauer *et al*. (2015) state that the administration of leucine-enriched whey protein for thirteen weeks can increase the muscle mass and lower the limb function in elderly community with sarcopenia.

Againts the backdrop above, this study aimed to analyse leucine intake as a determinant of muscle strength and gait speed. The results of this study will help set a basis for developing further epidemiological research in the prevention of sarcopenia.

METHODS

Design, location, and time

This observational study used a cross–sectional design. The study was conducted in Girimaya Subdistrict, the working area of Girimaya Public Health Center, Pangkalpinang City, in October 2017–May 2018.

Sampling

The sample size was determined pourposively to represent all of the 5 (five) Integrated Development Posts in 5 (five) villages in Girimaya Subdistrict. Screening was performed on the potential study participants based on the inclusion and exclusion criteria, resulting in a total of 132 participants. The inclusion criteria were that the participants were elderly people aged 60–69 years, able to walk independently without tools, not staying in an elderly institution, able to grasp, and willing to participate in the study and to be cooperative. The exclusion criteria were that the participants were suffering from Alzheimer's disease, diabetes mellitus, severe cardiovascular, and/or muscle problems, and were undergoing hospitalization. The exclusion criteria were established based on the studies by Li *et al*. (2015) and Cruz-Jentoft *et al*. (2010), which state that chronic diseases, including organ failure, inflammatory factors, endocrine disease, diabetes, hypertension, arthritis, and osteoporosis, are associated with low muscle strength and physical performance.

Data were collected after approval was obtained from the Health Research Ethics Commission of the Faculty of Medicine of Diponegoro University with certificate number 19/EC/FK-RSDK/I/2018. Prior to data collection, each participant was asked for consent to the study procedures and signed the informed consent sheet.

Data collection

The independent variables were the intakes of leucine amino acid, protein, and energy, while the dependent variables were muscle mass, muscle strength, and gait speed.

Muscle mass measurement was performed by Bioelectrical Impedence Analysis (BIA), using the digital body fat monitor FEP-103 (Oserio). The calculation of the muscle mass index was performed by dividing the muscle mass in kilograms by the square of height in meters (muscle mass/height^2); measurement of muscle strength was performed with Camry digital hand dynamometer (EH101), three times on the dominant hand in a standing position, the highest value taken; measurement of gait speed was performed for 6 minutes by the American Thoracic Society (ATS) procedures (ATS 2012; Li *et al.* 2013). Mileage was calculated based on the ability of the subject during that period to determine the gait speed in meters per second; analysis of food intake was carried out using the recall method 1 x 24 hours for 3 consecutive days by recording the types and amounts of food consumed over the past 24 hours.

The study used the BIA method because it is reliable and easy to use. Hand grip strength (HGS) is a gold standard and is easier to use in the study of the community. Meanwhile, gait speed was also chosen due to the ease of implementation (Naseeb & Volpe 2017). The Taiwan population

cut-off points were chosen as indicators due to the resemblance in anthropometric characteristics and life expectancy with those of the elderly in Indonesia in terms of ethnicity, genetics, body size, lifestyle, and cultural background. Therefore, the indicators for muscle mass assessment were <8.87 kg/m^2 for men and <6.42 kg/m^2 for women, the indicators for muscle strength assessment were <22.5 kg for men and <14.5 kg for women, and the indicator for gait speed of an elderly was ≤1 m/sec (Vitriana *et al*. 2016; Chen *et al*. 2016; Chen *et al*. 2014).

Data analysis

All data were subjected to processing through editing, coding, and tabulation. Data on gender, age, body weight, body height, body mass index, muscle mass, muscle mass index, muscle strength, gait speed, protein, leucine, and energy intake were presented descriptively. It was followed by bivariate and multivariate analysis to measure the correlation between the independent and dependent variables as well as the diffrences between groups in the multivariate model. The bivariate analysis used the Pearson's correlation test with a significance value of $p<0.05$. The multivariate analysis used multiple linear regression tests to test the variables; protein, leucine, and energy intake with a p value <0.25. The analysis were carried out using IBM SPSS Statistics version 21.

RESULTS AND DISCUSSION

The participants were elderly people in the community, aged 60–69 years with characteristics presented in Table 1. General description and the study variables are shown in Table 2.

Subject characteristics. The majority of the study participants were women (74.2%). It is known that sex, is one of the factors that can influence muscle strength and physical performance in the elderly. The study of Zeng *et al*. (2016) shows that the decrease in gait speed among the elderly was more common in women (40.69%) than in men (28.67%), whereas the decreased in muscle strength was more common in men (34.58%) than in women (28.54%). The average age of the elderly was 63 years, with the lowest age being 60 years and the highest 69 years. Age is also one of the factors that can affect muscle strength and physical performance in the elderly. Zeng *et al*. (2016) study states that the decreases in gait speed and muscle strength occurred after the age of 60 years in the elderly population in China. There are significant differences in muscle strength and gait speed based on age group.

Table 1. Characteristics of participants (n=132)

Variable	n	%
Gender		
Male	34	25.8
Female	98	74.2
Nutritional status (BMI*)		
Low (<18.5 kg/m^2)	9	6.8
Normal (≥18.5 – <24.9 kg/m^2)	56	42.5
Obese (≥25 kg/m^2)	67	50.7

BMI: Body mass index (Report of Riskesdas 2013)

General description and study variables. More than half of the participants were obese (50.7%). Obesity and unstructured daily activities were associated with increased risk of lower gait speed with OR (95% CI): 1.25 (1.09–1.43) and 2.77 (1.34–5.72) (Zeng *et al*. 2016). Obesity synergistically causes metabolic disorders and affects physical function, especially in the female elderly population in Asia (Wu *et al*. 2016). Obesity and sarcopenia are double burdens of health problem commonly found in the elderly who are less physically active, and further, this condition can lead to an increase in risk of insulin resistance, dyslipidemia, heart disease, and metabolic diseases (Bauer *et al*. 2015; Deutz *et al*. 2014; Naseeb & Volpe 2017). The frequency distribution of assessment of sarcopenia can be seen in Table 3.

Muscle mass index. It was known that the mean of muscle mass and muscle mass index of the elderly were respectively 35.1 kg and 15.6 kg/m² (see Table 2). The lowest muscle mass index was 10.07 kg/m^2, and the highest 20.20 kg/m^2, meaning that even the lowest value has exceeded the normal cut-off for the Taiwan population. The study by Vitriana *et al*. (2016) used the cut-off of the Taiwan population to determine the prevalence of sarcopenia in the elderly (60–85 years) in Bandung.

Table 3 describes that the muscle mass index of the elderly participating in this study was all in the moderate category (100%), so the diagnosis of sarcopenia could not be established based on the cut-off for the Taiwanese population. The process of sarcopenia is characterized by

Table 2. General description and study variables (n=132 people)

Variable	Mean±SD	Median	Min-Max	p value
Age (years)	-	63	60–69	0.000
Weight (kg)	58.7±11.8	-	28.2–92.1	0.200
Height (m)	1.5±0.1	-	1.4–1.7	0.062
Body mass index (kg/m^2)	25.3±4.6	-	13.6–37.9	0.200
Muscle mass (kg)	-	35.1	22.7–53.6	0.000
Muscle mass index (kg/m^2)	15.6±1.5	-	10.1–20.2	0.200
Muscle strenght (kg)	19.6±7.3	-	6.6–45.3	0.060
Gait speed (m/s)	0.9±0.2	-	0.3–1.5	0.200
Protein intake (g)	48.2±1.4	-	23.6–88.	0.200
Leucine intake (mg)	3,820±1,092	-	1,866,7–7,200	0.055
Energy intake (kcal)	1.01±328	-	678.6–2,273,2	0.200

SD: Standard deviation; Kolmogorov-Smirnov test; p value>0.05 (normally distributed)

a lack of muscle mass index, coupled with low muscle strength or low physical performance (Cruz-Jentoft *et al*. 2010). The normal muscle mass index found in the participants was thought to correlate with the energy and leucine amino acid intake.

However, in early 2018, the European Working Group on Sarcopenia in Older People (EWGSOP2) revised the diagnosis of sarcopenia with the aim of raising awareness of sarcopenia and risk factors as we get older. Sarcopenia identification focuses on low muscle strength as the main characteristic, then detects low muscle quantity and quality, and poor physical performance as an indication of severe sarcopenia. Based on EWGSOP2 recommendations, it was known that the prevalence of elderly sarcopenia based on the decrease of muscle strength was 29.6% (7.6% in men and 22% in women) (Cruz-Jentoft *et al*. 2019).

Muscle strength. The average muscle strength of the elderly was 19.6 kg. The study by Vitriana *et al*. (2016) used the cut-off of the Taiwan population to determine the prevalence of sarcopenia in the elderly (60–85 years) in Bandung. The criteria for elderly muscle strength in China are determined based on recommendations of the Asian Working Group for Sarcopenia (Zeng *et al*. 2016).

Based on the study by Vitriana *et al*. (2016), the main factors that influence the hand grip strength of the elderly are age, gender, total body fat, and fat-free mass. The factors age, gender, life style, and health status can affect the muscle strength of the elderly (Zeng *et al*. 2016). The study by Zeng *et al*. (2016) states that the correlation between muscle strength and physical performance varies according to age categories.

Gait speed. The average gait speed of the elderly was 0.9 m/sec. The study by Vitriana *et al*. (2016) used the cut-off of the Taiwan population to determine the prevalence of sarcopenia in the elderly (60–85 years) in Bandung. Zeng *et al*. (2016) used the recommendations of the Asian Working Group for Sarcopenia (AWGS) in determining the criteria for elderly gait speed in China.

Protein intake. The average protein intake of the elderly was 48.2 g, which was lower than the national average of 61 g based on RDA 2013 (BKPM 2013). Hence, low protein intake is one of the risk factors for sarcopenia in the elderly (Deutz *et al*. 2014). Sarcopenia interventions can be done by consumption of foods high protein and exercising (Wu *et al*. 2016).

Leucine intake. The average leucine amino acid intake of the elderly was 3,820 mg (3.8 g) per day. The leucine source foods consumed by the elderly included: sea fish, white rice, bread/cake, tempeh, tofu, eggs, chicken, meat and vegetables. The need for the amino acid leucine of the elderly was calculated as 39 mg/kg weight/day. The World Health Organization (WHO) states that the estimated leucine requirement was 39 mg/kg per day or 59 mg/g of protein per day (Report of FAO/WHO/UNU 2002).

Energy intake. Energy intake was obtained from carbohydrates (rice, bread, tubers), proteins (sea fish, meat, chicken, eggs, tofu, tempeh), fats (oil, coconut milk), and vitamins (vegetables, fruits). Table 4 describes the correlation test of the amino acid leucine, protein, and energy intake to muscle strength and gait speed.

Table 3. Frequency distribution of assessment of sarcopenia (n=132)

Assessment of sarcopenia	Male		Female	
	n	%	n	%
Muscle mass index (kg/m^2)				
Moderate/good (male: ≥8.87; female: ≥6.42)	34	25.8	98	74.2
Less (male: <8.87; female: <6.42)	0	0	0	0
Muscle strength (kg)				
Moderate/good (male: ≥22.5; female: ≥14.5)	24	18.2	69	52.2
Less (male: <22.5; female: < 14.5)	10	7.6	29	22
Gait speed (m/s)				
Moderate/good (male and female: >1)	20	15.1	17	12.9
Less (male and female: ≤1)	14	10.6	81	61.4

Leucine amino acid intake was positively correlated with muscle mass index ($p<0.05$), muscle strength ($p<0.001$), and gait speed ($p<0.05$). The elderly who had essential amino acid intake for 16 weeks experienced an increase in muscle mass and its function without any exercise intervention (Deutz *et al*. 2014). Proper nutrition intake, especially energy and protein, and physical activity have a role in the management of sarcopenia (Naseeb & Volpe 2017; Kim *et al*. 2010; Arnold & Bautmans 2014). Verhoeven *et al*. (2009) show that only long-term sources of leucine do not increase muscle mass or muscle strength.

Protein intake was positively correlated with muscle mass index ($p<0.001$), muscle strength ($p<0.001$), and gait speed ($p<0.05$) in the elderly in the community. The average protein intake of the elderly was 48.2 g, which was lower than the national average of 61 g based on RDA 2013 (BKPM 2013). Deutz *et al*. (2014) state that when the daily protein intake cannot fulfill the increase in protein requirements of the elderly to maintain muscle function, negative nitrogen balance will occur and a decrease in protein levels, especially skeletal muscle protein happens. The habit of consuming the right amount of protein can reduce the loss of muscle function and inhibit the occurrence of sarcopenia in the elderly. Kobayashi *et al*. (2013) found that a total protein intake of (74±14.3 g/day) had a significant association with muscle susceptibility ($p<0.05$), regardless of source and type of amino acid consumed. Tieland *et al*. (2012) assessed that the dietary intake of protein sources, daily protein distribution, and use of food sources containing protein (such as bread and milk) in community-dwelling (n=707), frail (n=194), and institutionalized (n=276) elderly people, could delay the occurrence of sarcopenia. Data of dietary intake of elderly people of living in the community, frail, and institutionalized in the Netherlands was collected based on the food records method for 2 to 3 days.

Energy intake was positively correlated with the sarcopenia process based on muscle mass index, muscle strength, and gait speed. The energy intake in the majority of the elderly was in the moderate category (1,301 kcal) in 97.7% of subjects. Arnold and Bautmans (2014) state that proper nutritional intake, especially energy and protein, and physical activity had a role in sarcopenia management.

One of determine the relationship between nutritional intake and skeletal muscle mass is age. In addition, an increased skeletal muscle mass is associated with greater energy intake. Generally, men eat more sources of calories, so

Table 4. Correlation test of leucine amino acid, protein, and energy intake to muscle strength and gait speed in elderly people in community

Variable	Muscle mass index		Muscle strenght		Gait speed	
	p value	r value	p value	r value	p value	r value
Leucine intake[a]	0.001*	0.299	0.000**	0.31	0.005*	0.244
Protein intake[b]	0.000**	0.315	0.000**	0.325	0.004*	0.251
Energy intake[c]	0.000**	0.375	0.000**	0.351	0.003*	0.255

a, b, c: Pearson's correlation coefficient; p value<0.001**: very significant; p value<0.05*: significant; r value: direction of correlation (positive)

there is a significant relationship between age and energy intake. Whereas in women less because it is influenced by patterns of age distribution and physiological changes (Jang & Bu 2018). The multiple linear regression analysis of energy, leucine amino acid, and protein intake can be seen in Table 5.

Leucine intake was not the most influential factor in the muscle mass index, muscle strength, and gait speed of the elderly. The average leucine amino acid intake of the elderly was 3.8 g per day. However, the amount could still influence the effectiveness of amino acid functions in maintaining muscle mass, muscle strength, and gait speed of the elderly. Based on the study by Verhoeven *et al*. (2009), leucine supplementation of 7.5 g per day for 3 (three) months was not effective in increasing the muscle mass or muscle strength of healthy elderly men. Total protein intake according to adequacy needs to be considered because it is related to amino acids. During the 3 months be seen the impact of leucine supplementation on skeletal muscle mass, strength, and/or glycemic control in healthy elderly men. Leucine supplementation could increase the upper leg cross-sectional area and change the type composition of muscle fiber, but did not affect the whole-body's fat-free mass and glycemic control and/or blood lipid profile.

Maintaining mobility, physical function, and independence of the elderly is important to inhibit the development of sarcopenia (Bauer *et al*. 2015). Physical activity and combination of nutritional intake, especially protein and energy, based on the recommended dietary allowance (RDA) can increase muscle mass, and physical function, and achieve a balance of positive muscle protein (Naseeb & Volpe 2017; Morley *et al*. 2010). The condition of sarcopenia or the risk factors for the cause of sarcopenia are not a barrier for the elderly to conduct physical activity (Liu *et al*. 2014).

Metabolic changes in the elderly cause less muscle protein synthesis despite the same amount of protein consumed by younger population (Morley *et al*. 2010). Protein food sources should be properly distributed, at a minimum of 25 to 30 g of high-quality protein per food item containing 2.5 to 2.8 g of leucine to stimulate muscle protein synthesis for the blunted sensitivity of older muscles to low doses of amino acids (Bauer *et al*. 2015). The quantity, quality, and time of protein intake are important factors for maintaining muscle mass and function (Bauer *et al*. 2015; Tieland *et al*. 2012).

The muscle mass and muscle strength in the elderly can be increased by intake high protein. Protein consumption of 1.1 g/kgW/day can affect lean body mass (LBM) stronger than protein consumption of 0.7–0.9 g/kgW/day. Protein intake between 1.1 and 1.6 g/kgW/day is needed to achieve nitrogen balance (Deutz *et al*. 2014). The consumption of whey protein can increase muscle protein synthesis better than casein. Whey protein intake contains enough leucine amino acids in protein synthesis to maintain muscle mass and function in the elderly with sarcopenia (Bauer *et al*. 2015). The

Table 5. Anylsis of multiple linear regression analysis of energy, leucine amino acid, and protein intake on muscle mass index, muscle strength, and gait speed

Variable	Constant value	Regression coefficient	Correlation coefficient	Anova test	Adj R square
Muscle mass index					
Leucine intake	-	-	-	-	-
Protein intake	-	-	-	-	-
Energy intake[a]	13.358	0.002	0.372	0.000	0.132
Muscle strength					
Leucine intake	-	-	-	-	-
Protein intake	-	-	-	-	-
Energy intake[a]	8.819	0.007	0.318	0.000	0.162
Gait speed					
Leucine intake	-	-	-	-	-
Protein intake	-	-	-	-	-
Energy intake[a]	0.747	0.0001	0.239	0.000	0.195

[a]: Variable that appeared at the end of the multiple linear regression test

pattern of protein intake which varies in 4 times of administration can stimulate protein synthesis in the elderly, which is affected by differences in age and exercise. Bollwein *et al*. showed that the amount of protein intake was not associated with frailty, but the distribution in the morning, afternoon, and evening in a way was associated with vulnerability (Kobayashi *et al*. 2013).

CONCLUSION

Protein and leucine amino acid intakes were positively correlated with muscle mass index, muscle strength, and gait speed in the elderly in the community. Leucine food intake was not a major determinant for muscle mass, muscle strength, and gait speed. Energy intake was the most influential factor in muscle mass, muscle strength, and gait speed. The elderly in the community are expected to increase food intake of energy, protein, and leucine amino acid to increase muscle mass, muscle strength, and gait speed.

ACKNOWLEDGEMENT

Thanks are due to BPPSDM of Ministry of Health of the Republic of Indonesia for the fund provided based on the Decree of the Minister of Health of the Republic of Indonesia Number HK.02.02/H.V/SK/169/2016 on Participants of Study on Health Human Resources and Assistance for Study in 2016, as well as nutrition workers and cadres at the Girimaya Community Health Center in Pangkalpinang City. The authors have no conflict of interest.

REFERENCES

[ATS] American Thoracic Society. 2002. ATS Statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 166(1):111–117. DOI: 10.1164/rccm.166/1/111.

Arnold P, Bautmans I. 2014. The influence of strength training on muscle activation in elderly persons: A systematic review and meta-analysis. Exp Gerontol 58:58–68. http://dx.doi.org/10.1016/j.exger.2014.07.012.

[BKPM] Badan Koordinasi Penanaman Modal. 2013. Angka kecukupan gizi yang dianjurkan bagi bangsa Indonesia. https://peraturan.bkpm.go.id/jdih/userfiles/batang/PMK%20No.%2075%20ttg%20Angka%20Kecukupan%20Gizi%20Bangsa%20Indonesia.pdf. [Accessed 20 March 2014].

Bauer JM, Verlaan S, Bautmans I, Brandt K, Donini LM, Maggio M, McMurdo MET, Mets T, Seal C, Wijers SL, *et al*. 2015. Effects of a vitamin D and leucine-enriched whey protein nutritional supplement on measures of sarcopenia in older adults, the Provide Study: A randomized, double-blind, placebo-controlled trial. Journal Jamda 16(9):740–747. http://dx.doi.org/10.1016/j.jamda.2015.05.021.

Chen LK, Lee WJ, Peng LN, Liu LK, Arai H, Akishita M, Asian Working Group for Sarcopenia. 2016. Recent advances in sarcopenia research in Asia: 2016 update from the Asian working group for sarcopenia. Jamda 17(8):767e1-767e7. https://doi.org/10.1016/j.jamda.2016.05.016.

Chen LK, Liu LK, Woo J, Assantachai P, Auyeung TW, Bahyah KS, Chou MY, Chen LY, Hsu PS, Krairit O, *et al*. 2014. Sarcopenia in Asia: Consensus report of the Asian Working Group for Sarcopenia. Jamda 15(2):95–101. doi: 10.1016/j.jamda.2013.11.025.

Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, Martin FC, Michel JP, Rolland Y, Schneider SM, *et al*. 2010. Sarcopenia: European consensus on definition and diagnosis: Report of the European working group on sarcopenia in older people. Age and ageing 39(4):412–423. doi: 10.1093/ageing/afq034.

Deutz NEP, Bauer JM, Barazzoni R, Biolo G, Boirie Y, Bosy-Westphal A, Cederholm T, Cruz-Jentoft A, Krznaric Z, Nair KS, *et al*. 2014. Protein intake and exercise for optimal muscle function with aging: Recommendations from the ESPEN expert group. Clinical Nutrition 33(6):929–936. http://dx.doi.org/10.1016/j.clnu.2014.04.007.

Gropper SS, Smith JL. 2013. Advanced nutrition and human metabolism. Sixth Edition. USA: Yolanda Cossio.

Jang BY, Bu SY. 2018. Total energy intake according to the level of skeletal muscle mass in Korean adults aged 30 years

and older: An analysis of the Korean national health and nutrition examination surveys (KNHANES) 2008–2011. Nutr Res Pract 12(3):222–232. doi:10.4162/nrp.2018.12.3.222.

Kim JS, Wilson JM, Lee SR. 2010. Dietary implications on mechanisms of sarcopenia: Roles of protein, amino acids and antioxidants (reviews). J Nutr Biochem 21(1):1–13. doi:10.1016/j.jnutbio.2009.06.014.

Kobayashi S, Asakura K, Suga H, Sasaki S. 2013. High protein intake is associated with low prevalence of frailty among old Japanese women: A multicenter cross-sectional study. Nutr J 12:164.

Li CI, Li TC, Lin WY, Liu CS, Hsu CC, Hsiung CA, Chen CY, Huang KC, Wu CH, Wang CY, *et al*. 2015. Combined association of chronic disease and low skeletal muscle mass with physical performance in older adults in the sarcopenia and translational aging research in Taiwan (START) study. BMC Geriatrics 15:11. DOI 10.1186/s12877-015-0011-6.

Liu CK, Leng X, Hsu FC, Kritchevsky SB, Ding J, Earnest CP, Ferrucci L, Goodpaster BH, Guralnik JM, Lenchik L, *et al*. 2014. The impact of sarcopenia on a physical activity intervention: the lifestyle interventions and independence for elders pilot study (LIFE-P). J Nutr Health Aging 18(1):59–64. doi:10.1007/s12603-013-0369-0.

Liu LK, Lee WJ, Liu CL, Chen LY, Lin MH, Peng LN, Chen LK. 2013. Age-related skeletal muscle mass loss and physical performance in Taiwan: Implications to diagnostic strategy of sarcopenia in Asia. Geriatr Gerontol Int 13(4):964–971. https://doi.org/10.1111/ggi.12040.

Morley JE, Argiles JM, Evans WJ, Bhasin S, Cella D, Deutz NEP, Doehner W, Fearon KCH, Ferrucci L, Hellerstein MK, *et al*. 2010. Nutritional recommendations for the management of sarcopenia. Jamda 11(6):391–396. doi:10.1016/j.jamda.2010.04.014.

Naseeb MA, Volpe SL. 2017. Protein and exercise in the prevention of sarcopenia and aging. Nutr Res 4:1–20 doi:10.1016/j.nutres.2017.01.001.

Report of a joint FAO/WHO/UNU expert consultation. Protein and amino acid requirements in human nutrition. 2002; WHO technical report series; 935. Geneva, Switzerland. p. 135-151.

Siswanto, *et al.* 2014. Studi Diet Total: Survei Konsumsi Makanan Individu Indonesia 2014, edisi pertama. Jakarta: Lembaga Penerbitan Badan Penelitian dan Pengembangan Kesehatan Kemenkes.

Tieland M, Berg KJBVD, Loon LJCV, De Groot LCPGM. 2012. Dietary protein intake in community-dwelling, frail, and institutionalized elderly people: Scope for improvement. Eur J Nutr 51(2):173–179. doi:10.1007/ s00394-011-0203-6.

Triatmaja NT, Khomsan A, Dewi M. 2013. Asupan kalsium, status gizi, tekanan darah dan hubungannya dengan keluhan sendi lansia di panti werdha Bandung. Jurnal Gizi dan Pangan 8(1): 25–32. DOI: https://doi.org/10.25182/jgp.2013.8.1.25-32.

Vitriana, Defi IR, Nugraha GI, Setiabudiawan B. 2016. Prevalensi sarkopenia pada lansia di komunitas (community dwelling) berdasarkan dua nilai cut-off parameter diagnosis. MKB 48(3): 164–170. https://doi.org/10.15395/mkb.v48n3.417.

Verhoeven S, Vanschoonbeek K, Verdijk LB, Koopman R, Wodzig WK, Dendale P, Loon LJCV. 2009. Long-term leucine supplementation does not increase muscle mass or strength in healthy elderly men. Am J Clin Nutr 89(5):1468–1475. https://doi.org/10.3945/ajcn.2008.26668

Wu YH, Hwang AC, Liu LK, Peng LN, Chen LK. 2016. Sex differences of sarcopenia in Asian populations: The implications in diagnosis and management. J Clin Gerontol Geriatr 7(2):37–43.

Yu SCY, Khow KSF, Jadczak AD, Visvanathan R. 2016. Clinical screening tools for sarcopenia and its management, review article. Curr Gerontol Geriatr Res 2016:1–10. http://dx.doi.org/10.1155/2016/5978523.

Zeng P, Han Y, Pang J, Wu S, Gong H, Zhu J, Li J, Zhang T. 2016. Sarcopenia-related features and factors associated with lower muscle strength and physical performance in older Chinese: A cross sectional study. BMC Geriatrics 16(1):45 DOI 10.1186/s12877-016-0220-7.

Available online: http://journal.ipb.ac.id/index.php/jgizipangan ***J. Gizi Pangan, March 2020, 15(1):27-36***
Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019 *DOI: 10.25182/jgp.2020.15.1.27-36*
ISSN 1978-1059 EISSN 2407-0920

Alternative Snack for Diabetic Patients from Sago (*Metroxylon Sp*.) Starch and Tempeh

Sofya Maya[1], Ahmad Sulaeman[1*], Tiurma Sinaga[1]
[1]Departement of Community Nutrition, Faculty of Human Ecology, IPB University, Bogor 16680, Indonesia

ABSTRACT

This study aimed to develop food products made from sago starch (*Metroxylon* sp.) and tempeh as alternative snacks for diabetics. This study used a completely randomized design (CRD) with two treatment ratios of sago starch to tempeh, F1 (2:1) and F2 (1:1), and two replications for three products, namely puddings, muffins, and cookies. The organoleptic attributes of the products were evaluated by fourty semi-trained panelists. The results of the hedonic test showed that F2 was the formula selected for the puddings and muffins and that F1 was the formula selected for the cookies. The puddings had hardness of 83.47 gf, 92.28% water, 0.28% ash, 1.04% protein, 0.64% fat, 5.76% carbohydrate, 3.95% dietary fiber, and 0.81% resistant starch. The muffins had hardness of 3,861,87 gf, 56.18% water, 0.79% ash, 6.49% protein, 10.26% fat, 26.28% carbohydrate, 7.13% dietary fiber, and 3.59% resistant starch. The cookies had hardness of 1,655,02 gf, 5.05% water, 0.90% ash, 4.90% protein, 19.66% fat, 69.49% carbohydrate, 9.57% dietary fiber, and 6.00% resistant starch. These products contained different levels of dietary fiber and resistant starch. Puddings are categorized as a high-fiber food with a negligible level of resistant starch, the muffin as a high-fiber food with an intermediate level of resistant starch, and the cookie as a high-fiber food with a high level of resistant starch. In conclusion, these products had relatively high fiber content and need a further study to confirm the health benefits for glucose control in diabetics.

Keywords: diabetes mellitus, dietary fiber, sago, snack, tempeh

INTRODUCTION

Type 2 diabetes mellitus (DMT2) is a complex metabolic disorder with both short- and long-term undesirable complications (Mirmiran *et al*. 2014). If it is not managed properly, it will cause various organ damages (Deepa *et al*. 2014) manifested in many types of health problems. In addition to proper medical care, diet regulation, including that of snacks, can be used in managing DMT2.

Main foods and snacks are needed by diabetics to control the blood glucose levels. The food ingredients for diabetics should be high in fiber content and low in glycemic index. Scheduling for snack is between two main meal times. It aims to meet adequate calorie intake and nutritional needs, prevent hypoglycemia that usually occurs at night, achieve or maintain normal weight, and control blood glucose in an effort to prevent the risk of complications in diabetics (Almatsier 2010).

A meta-analysis study by Post *et al*. (2012) showed that increased fiber consumption (4–40 mg/day) could reduce fasting blood glucose and HbA1c in patients with type 2 diabetes by 0.05 mmol/L and 0.26%, respectively. Resistant starch, which is part of fiber, can also control blood glucose in the diabetic rats given an intervention of resistant starch of 2 g/day for 4 weeks (Zhou *et al*. 2015).

Several studies related to snack development for diabetics with the use of various high-fiber, low-glycemic-index ingredients have been carried out, such as those developing black rice and black soybean flour cookies (Widiawati & Anjani 2017), bagel bread from the substitution of sorghum and sweet potatoes (Ashfiyah 2019), and pudding made from corn starch and tapioca (Gourineni *et al*. 2017). However, sago starch is rarely developed as snack for diabetics despite the fact that sago starch (Metroxylon sp.) contains 3.69–5.96% dietary fiber (Ahmad *et al*. 1999) and 11% resistant starch (Purwani 2011) and has a low glycemic index (<55) (Wahjuningsih *et al*. 2016).

The local ingredient sago starch has a potential to be developed to be snacks

[*]**Corresponding Author:** tel: +6285775264968, email: asulaema06@gmail.com

for diabetics. In addition to sago, as stated by Rudkowska (2009), beneficial additional sources in food products for diabetics will be able to help control blood glucose. Therefore, tempeh, which has been proven to improve blood glucose, will be added in the making of food products (Ghozali *et al*. 2010). Meanwhile, food processing by roasting, steaming, boiling, or burning is recommended for patients with type 2 diabetes with the aim of reducing calorie intake (Almatsier 2010). Therefore, puddings, steamed muffins, and cookies from sago starch and tempeh offer some options for the development of snacks for patients with type 2 diabetes.

This study aimed to explore the nutrient content, dietary fiber content, and resistant starch content of puddings, steamed muffins, and cookies made from sago strach and tempeh to confirm the benefits of the products.

METHODS

Design, location, and time

This study used a completely randomized design (CRD) with two treatment ratios of sago starch to tempeh, F1 (2:1) and F2 (1:1), with two replications for three products. The products' formulation was conducted in the Processing and Food Experiment Laboratory of the Department of Community Nutrition, IPB University. Sensory evaluation was conducted in the Organoleptic Laboratory of the Department of Community Nutrition, IPB University. Product texture analysis was conducted in the Food Processing Laboratory of the Department of Food Science and Technology, IPB University. The analysis of nutritional and fiber content was conducted in the Laboratory of Saraswanti Indo Genetech, Bogor. Analysis of resistant starch was conducted in the Biochemistry Laboratory of the Department of Food Science and Technology, IPB University. The study was conducted in March–October 2018.

Materials and tools

The main materials used to manufacture puddings, muffins, and cookies were sago starch derived from the Meranti Islands Regency, Riau, and tempeh obtained from Rumah Tempe Indonesia. The additional ingredients used were pumpkin of the bokor variety (*Cucurbita moschata* Durch), sorbitol and erythritol, full cream milk, chicken eggs, margarine, baking soda, baking powder, carrageenan, vanilla powder, salt, succade cherry, water, and cinnamon powder. The materials for the proximate analysis were selenium, H2SO4, distilled water, 40% NaOH, 2% boric acid (H3BO3), methyl red indicator, 0.1 N HCl, and hexane. The materials for the dietary fiber analysis were pH 8.2 buffer solution, α-amylase, protease, HCl, amyloglucosidase, 95% ethanol, and 78% acetone. The materials for the resistant starch analysis were KCl-HCl buffer, pepsin, phosphate buffer, α-amylase, KOH, HCl, and sodium acetate buffer.

The tools used to make puddings, muffins, and cookies were pans, oven, pudding and muffin cups, and cookie molds. The analytical tools were TA-XT2i texture analyzer, oven, Kjeldahl flask, and Soxhlet extractor.

Procedures

Product preparation. The foods product formulation (Table 1) was based on the calculation of the nutritional need of diabetics per Perkeni (2015). Assuming that adult women would measure 51 kg in weight and 155 cm in height, the energy need would be 1,700 calories/day. The main limitation in determining the product formula was the carbohydrate content of foods (45–65% of total energy requirements) (Perkeni 2015). The complete formula is under registration for patent.

The foods developed in this study were puddings, muffins, and cookies. The preparation of ingredients such as sago starch, tempeh, and pumpkin was performed in the same way, but the processing was different for each food type. Sago starch was roasted first with an addition of pandan leaves for reduced acid aroma. Tempeh and pumpkin were cut to a thickness of ±0.2 cm and steamed for 10 minutes, then mashed.

Pudding. Pudding processing began with determining the amount of water and carrageenan used. The proportion of water to sago starch in the pudding making was 20:1, while the carrageenan used amounted 1% of the total weight. The pudding was processed by boiling to ±80^{o}C until the dough became thick.

Muffin. The muffin formula was modified from Kementan (2012). The first step was to mix eggs, sorbitol, and erythritol using a hand mixer at a medium speed until the mixture turned white. Next, tempeh, pumpkin, and some other

Table 1. Formulation of puddings, muffins, and cookies from sago starch and tempeh

Materials	Comparison of sago strach and tempeh					
	Pudding		Muffin		Cookies	
	2:1	1:1	2:1	1:1	2:1	1:1
Sago strarch (g)	380	190	320	160	350	175
Tempeh (g)	190	190	160	160	175	175
Pumpkin (g)	180	180	150	150	150	150
Sorbitol and erythritol (g)	80	80	80	80	80	80
Full cream milk (ml)	150	150	150	150	50	50
Chicken egg (g)	-	-	150	150	-	-
Egg yolk (g)	-	-	-	-	20	20
Margarine (g)	-	-	100	100	150	150
Carrageenan (g)	85.81	45.91	-	-	-	-
Water (ml)	7,600	3,800	-	-	-	-
Miscellaneous (g)	3	3	4.8	4.8	27	27

ingredients were added into the mixture using the hand mixer at a low speed. The next step was to put some dough into muffin cups, which was then steamed for 30 minutes at 100°C.

Cookie. The cookies formula was modified from Saputri and Damayanti (2015). The process of cookie making consisted of several stages, namely weighing ingredients, mixing, molding of dough, baking with an oven at 120°C for 80 minutes, and cooling.

Sensory evaluation. The formula selected for the three products was determined based on the results of the hedonic test analysis. Sensory evaluation was conducted on 40 semi-trained panelists, who were students of the Department of Community Nutrition, Faculty of Human Ecology, IPB University. Sensory attributes analysis of the three products was conducted using a line scale. The scale used in the hedonic test was as follows: (1) dislike extremely, (2) dislikes very much, (3) dislikes moderately, (4) dislikes slightly, (5) neither dislike nor like, (6) like slightly, (7) like moderately, (8) like very much, and (9) like extremely. Panelists were considered to accept a product if the value given was greater than 4.5 (Peryam & Pilgrim 1957).

The nine-point quality hedonic scale for the pudding's color atribute was as follows: (1) pale white, (5) pale yellow, and (9) brownish yellow. The nine-point quality hedonic scale for the muffin's color atribute was as follows: (1) bright beige, (5) golden yellow, and (9) golden brown. The nine-point quality hedonic scale for the cookie's color atribute was as follows: (1) extremely brown, (5) beige, and (9) extremely yellow. The nine-point quality hedonic scale for unpleasant aroma and aftertaste atribute was as follows: (1) extremely weak, (5) normal, and (9) extremely strong. The nine-point quality hedonic scale for the sweetness atribute was as follows: (1) not sweet at all, (5) normal, and (9) extremely sweet.

The nine-point quality hedonic scale for the pudding's texture atribute was as follows: (1) chewy and extremely brittle, (5) normal, and (9) chewy and extremely elastic. The nine-point quality hedonic scale for the muffin's texture atribute was as follows: (1) extremely dense, (5) normal, and (9) extremely hollow. The nine-point quality hedonic scale for the cookie' texture atribute was as follows: (1) extremely brittle, (5) normal, and (9) crunchy.

Physicochemical analysis of selected products. The 74-09 method was employed for the analysis of product hardness (AACC 2001). The analysis of water content (gravimetric method), ash content (dry ashing method), protein content (micro-Kjeldahl method), and fat content (Soxhlet method) was based on SNI 01-3775-2006 (BSN 2006). The carbohydrate content was calculated by difference. The enzymatic method was used for the analysis of dietary fiber (AOAC 1995) and resistant starch (Goni *et al*. 1996).

Data analysis

Data processing was undertaken using Microsoft Excel 2010 and SPSS 15.0 for Windows. The data from the sensory evaluation were analyzed by a difference test of Mann Whitney with a 95% confidence interval to

determine the hedonic difference of each product. If the analysis result was $p<0.05$, the difference would be considered statistically significant.

RESULTS AND DISCUSSION

Panelists' acceptance of the sensory attributes of the puddings, muffins, and cookies from sago starch and tempeh

Hedonic test. The hedonic test was used to find out the panelists' responses about product likes or dislikes. The sensory attributes evaluated in the pudding hedonic test were color, taste, aroma, texture (surface press), chewing texture (mealiness), and aftertase. The sensory attributes evaluated in the muffin and cookie hedonic tests were color, taste, aroma, texture, and aftertaste.

The data in Table 2 show that the F2 pudding was the most acceptable product based on the color sensory attribute. The F2 formula used a ratio of sago starch to tempeh of 1:1. This study shows that a difference in the amount of sago used resulted in a significant difference in the panelists' preference in the pudding color ($p<0.05$). The average values of the panelists' preference in the color, taste, aroma, texture, and overall attributes of the F2 pudding were significantly higher than those of the F1 pudding. The average value of the panelists' preference in the color, taste, aroma, texture (press surface), chewing texture, aftertaste, and overall attributes of the F2 pudding were 6.2 (like slightly), 5.0 (neither dislike nor like), 6.1 (like slightly), 6.0 (like slightly), 5.3 (neither dislike nor like), 4.3 (dislike slightly), and 5.3 (neither dislike nor like), respectively.

The F2 muffin was the most acceptable product based on the sensory attributes (taste, texture, aftertaste, and overall) (Table 2). The F2 formula was used a ratio of sago starch to tempeh of 1:1. This study shows that a difference in the amount of sago used resulted in significant differences in the panelists' preference in the taste, texture, aftertaste, and overall attributes of the muffin ($p<0.05$). The average values of the panelists' preference in the color, taste, aroma, texture, and aftertaste of the F2 muffin were significantly higher than those of the F1 muffin. Increasing the amount of non-wheat flour in the muffin making would produce an increasingly dense texture. This would affect the level of preference for other attributes, resulting in reduced acceptance of the muffin (Goswami *et al*. 2015; Rismaya 2016).

The average values of the panelists' preference in the color, taste, aroma, texture, aftertaste, and overall attributes of the F2 muffin were 6.4 (like slightly), 6.6 (like slightly), 6.3 (like slightly), 6.2 (like slightly), 5.9 (neither dislike nor like), and 6.5 (like slightly), respectively.

The use of 100% sago flour and no wheat flour in the making of muffins was presumably the cause of the decrease in the value of the panelists' preference for muffins (like slightly). The high fiber and resistant starch content in sago would affect the amount of air trapped in the dough's matrix, causing the muffin softness to reduce or the texture to become rather dense (Struck *et al*. 2016). This would affect the panelists' preference for the muffins mainly in the texture attributes.

The data in Table 2 showed that the F1 cookie was the most acceptable product based

Table 2. Panelists' mean hedonic scales of the sensory attributes of the puddings, muffins, and cookies from sago starch and tempeh

Products		Sensory attributes						
		Colour	Taste	Aroma	Texture (surface press)	Chewing texture (mealiness)	After-taste	Over-all
Pudding	F1	4.8[a]	4.5	5.7	5.8	5.1	4.9	5.0
	F2	6.2[b]	5.0	6.1	6.0	5.3	4.3	5.3
Muffin	F1	5.4	5.2[a]	5.2	3.5[a]	-	4.8[a]	4.3[a]
	F2	6.4	6.6[b]	6.3	6.2[b]	-	5.9[b]	6.5[b]
Cookies	F1	6.4[a]	6.3	6.2	5.9[a]	-	5.6	6.3
	F2	6.5[b]	5.6	6.2	4.3[b]	-	5.4	5.4

Description: Attribute scales from 1 (dislike extremely) to 9 (like extremely); Different letters in the same column show significant differences ($p<0.05$)

on the sensory attributes (color and texture). The F1 formula used a ratio of sago starch to tempeh of 2:1. This study shows that an addition of sago resulted in significant differences in the panelists' preference in the attributes of color and texture of the cookie ($p<0.05$). The average values of the panelists' preference in the colour, taste, aroma, texture, aftertaste, and overall attributes of the F1 cookie were significantly higher than those of the F2 cookie. The dough structuring of the cookies was affected by the amount of water used in the formulation of the cookies (Norhidayah *et al*. 2014). The use of a small proportion of sago starch in the F2 cookie raised the water content of the cookie, leaving an effect on the texture (Budzaki *et al*. 2014) and decreasing the level of acceptance of the F2 cookie. The average values of he panelists' preference in the colour, taste, aroma, texture, aftertaste, and overall attributes of the F1 cookie were 6.4 (like slightly), 6.3 (like slightly), 6.2 (like slightly), 5.9 (neither dislike nor like), 5.6 (neither dislike nor like), and 6.3 (like slightly), respectively.

Hedonic quality test. The hedonic quality test was used to find out the panelists' responses based on good or bad impression of the puddings, muffins, and cookies from sago starch and tempeh.

The results of the hedonic quality test show that the puddings were yellowish white (F1) and yellow (F2) in color, the muffins golden yellow (F1) and yellow (F2), and the cookies beige (F1) and yellowish (F2) (Table 3). This study shows that a difference in the amount of sago used resulted in a significant difference in the muffins' color ($p<0.05$). The factors that might contribute to the color of the final products were the ingredient composition and cooking time (Cronin & Preis 2000). The muffins used cinnamon in the ingredient composition, while the puddings and cookies did not. This shows that the use of different amounts of sago and the use of cinnamon in making muffins would presumably produce different colors of the muffins. The use of cinnamon in conjunction with a greater amount of sago starch (F1 muffin) would produce less bright color than would the use of a smaller amount of sago starch (F2 muffin). Cinnamon can function as coloring agents in food (Hernández-Ochoa *et al*. 2011). The color of the puddings, muffins, and cookies itself was obtained from the pumpkin added as a natural food coloring.

The *langu* (unpleasant) aroma of the puddings, muffins, and cookies ranged from weak to rather weak. The process of steaming tempeh and pumpkin for 10 minutes was thought to reduce the unpleasant aroma of tempeh and pumpkin, causing the unpleasant aroma of the product to be weak and rather weak. The use of certain processing methods was one way to reduce the unpleasant aroma of food (Liu 1997), for example, by boiling or steaming. This study shows that the difference in the amount of sago used resulted in a significant difference in the muffins' unpleasant aroma ($p<0.05$).

The results of the hedonic quality test show that the puddings were not sweet (F1) and

Table 3. Panelists' mean hedonic quality scales of the sensory attributes of the puddings, muffins, and cookies from sago starch and tempeh

Products		Sensory attributes				
		Colour	*Langu* aroma	Sweetness	Texture	Aftertaste
Pudding	F1	4.0[a]	3.8	3.9[a]	5.1	5.1[a]
	F2	6.0[b]	4.0	5.3[b]	5.0	6.0[b]
Muffin	F1	5.3[a]	4.3[a]	5.1[a]	2.2[a]	5.1
	F2	4.1[b]	3.3[b]	6.1[b]	5.8[b]	4.8
Cookies	F1	5.5	3.5	6.0	5.5[a]	4.5
	F2	6.0	3.5	6.3	4.0[b]	4.8

Description: The overall pudding's colour of the scale 1=pale white to 9=brownish yellow; Muffin's colour scale 1=bright beige to 9=golden brown; Cookies' colour scale 1=brown extremely to 9=yellow extremely; *Langu* aroma and aftertaste scale 1=weak extremely to 9=strong extremely; Sweetness scale 1=not sweet at all to 9=sweet extremely; Pudding's texture 1=chewy and brittle extremely to 9=chewy and elastic extremely; Muffin's texture 1=dense extremely to 9=hollow extremely; Cookies' texture 1=brittle extremely to 9=crunchy; Different letters in the same column show significant differences ($p<0.05$)

normal (F2) in taste, the muffins normal (F1) and rather sweet (F2), and the cookies rather sweet (both F1 and F2) (Table 3). This study shows that the difference in the amount of sago used resulted in a significant difference in the sweetness of the puddings and muffins ($p<0.05$) but no significant difference in the sweetness of the cookies. Lesser amount of sago starch used in making the products would produce a sweeter taste. The sweetness of the cookies of the two formulas did not show any significant difference, but the F2 cookie had a higher sweetness value (6.3) than the F1 cookie (6.0).

The results of the hedonic quality test show that the puddings had a normal (both F1 and F2) texture, the muffins very dense (F1) and normal (F2), and the cookies normal (F1) and rather brittle (F2) (Table 3). This study shows that a difference in the amount of sago used resulted in a significant difference in the texture of the muffins and cookies ($p<0.05$). Sago starch as the main ingredient of the muffins is a source of non-gluten starch. The research by Rismaya (2016) shows that an increased amount of non-gluten flour results in a dense muffin texture. The gluten network retains the fermentation gas and determines the stability of gas cells during expansion, contributing soft (spongy) and flexible (elastic) crumbs and also influencing the characteristic appearance of bread products like muffins (Wrigley *et al*. 2006). In making cookies, the higher level of use of non-wheat flour will result in better texture and hardness of the cookies (Ajila *et al*. 2008). This study shows that a difference in the amount of sago used did not result in a significant difference in the texture of the puddings ($p>0.05$) due to the controlled amount use of water and carrageenan for each formula.

Aftertaste is the trace, hint, smack, relish, and savor food leaves behind (World Food and Wine 2005). The results of the hedonic quality test show that the puddings had normal (F1) and rather strong (F2) aftertaste, the muffins normal (F1) and rather weak (F2), and the cookies rather weak (both F1 and F2) (Table 3).

Percentage of panelists' acceptance

The formula for each product was chosen by considering the results of the sensory evaluation, namely the hedonic test, especially on the percentage of panelists' acceptance in the overall attributes and panelists' mean hedonic scales of the overall attributes.

Percentage of panelists' acceptance in the overall attributes of the F2 pudding (70.0%) and F2 muffin (91.2%) were significantly higher than the F1 pudding and F1 muffin (Table 4). Beside that, panelists' mean hedonic scales of overall attributes for the F2 pudding and F2 muffin were significantly higher than the F1 pudding and F1 muffin too (Table 2). Therefore, F2 was the selected formula for puddings and muffins. The F2 formula used a ratio of sago starch to tempeh of 1:1. The low level of acceptance of the F1 muffin was presumably caused by the use of a greater proportion of sago starch than that used in the F2 formula. An increased concentration of non-wheat flour would produce increasingly dense muffin textures, which caused a reduction in the level of acceptance (Goswami *et al*. 2015; Rismaya 2016).

The percentage of panelists' acceptance in the overall attributes of the F1 cookie (91.2%) was

Table 4. Percentage of panelists' acceptance on the sensory attributes of the puddings, muffins, dan cookies from sago starch and tempeh (%)

Products		Sensory attributes						
		Colour	Taste	Aroma	Texture (surface press)	Chewing texture (mealiness)	Aftertaste	Over-all
Pudding	F1	57.5%	45.0%	90.0%	85.0%	70.0%	56.2%	61.2%
	F2	88.7%	60.0%	90.0%	81.2%	66.2%	35.0%	70.0%
Muffin	F1	78.7%	70.0%	73.7%	18.7%	-	61.2%	33.7%
	F2	90.0%	90.0%	87.5%	86.2%	-	83.7%	91.2%
Cookies	F1	85.0%	88.7%	87.5%	76.2%	-	81.2%	91.2%
	F2	95.0%	75.0%	96.2%	31.2%	-	73.7%	72.5%

significantly higher than the F2 cookie (72.5%) (Table 4). Moreover, the panelists' mean hedonic scales of overall attributes for the F1 cookie was also significantly higher than the F2 cookie (Table 2). Therefore, F1 was the selected formula for cookies. The F1 formula used a ratio of sago starch to tempeh of 2:1. A smaller proportion of sago starch in the F2 cookie produced high water content, which inflenced the texture of the cookie (Budzaki *et al*. 2014) and resulted in a decrease in the level of acceptance of the F2 cookie.

Physical characteristics of selected products (hardness)

Hardness is one of the important indicators should be taken into account in analyzing food texture, and it has an impact on the level of consumer acceptance of the food products. The hardness value of the pudding was 83.47 gf. This value is lower compared to those of the puddings in other research, such as the mocaf dextrin pudding with hardness of 151.78–657.93 g/cm^2. The low strength of the pudding gel produces soft pudding (Darmawan *et al*. 2014). This means that the sago pudding's chewy but brittle texture was due to the low gel strength value. This result was presumably caused by the distinct proportion of the composition, including the carrageenan, in each pudding. An increased carrageenan concentration in the pudding mixture would increase the strength of the pudding gel (Trckova *et al*. 2004).

The hardness value of the muffins was 3,861,87 gf. Fiber content and resistant starch in non-wheat flour can produce a high muffin hardness value. Food fiber and resistant starch would affect the amount of air trapped in the dough matrix, reducing the muffin tenderness (Struck *et al*. 2016).

The hardness value of the cookies was 1,655,02 gf. The high amylose content (27%) in sago starch (Ahmad & Williams 1998) affected the hardness of the cookies (Horstmann *et al*. 2016). Higher amylose content would produce a good texture of cookies, but starch containing high amylopectin tends to produce breakable cookies (Claudia & Widjanarko 2016).

Nutrient content, dietary fiber, and resistant starch of selected products

The serving size of the sago pudding is 80 g (2 small cups), which refers to the food serving size for diabetics by Poetker (2010). A small cup of sago muffin weighs 38–39 g, which refers to the serving size of a muffin for diabetics by DHHS and USDA (2015) (1 small cup). One sago cookie weighs 6–7 g. The serving size for sago cookie is 20 g (3 pieces) as per the serving size of commercial cookies (sugar-free cookies). Nutrient content, dietary fiber, and resistant starch of pudding, muffin, and cookie per serving size are shown in Table 5.

The results in Table 5 show that the percentage of contribution of the puddings, muffins, and cookies (per serving size) as snacks to diabetics' needs (1,700 kcal) was very low and failed to meet the 10–15% needs of energy, protein, carbohydrate, fat, and dietary fiber. The puddings, muffins, and cookies per serving size consumed as daily snacks (2–3 times per day) will contribute more than 1.4 g of resistant starch. Lockyer and Nugent (2016) showed that a

Table 5. Nutrient content, dietary fiber, and resistant starch of selected puddings, muffins, and cookies from sago starch and tempeh

Components	Nutrient content per serving			Diabetics' needs*	Nutritional needs of snack (10-15%)	Contribution to nutritional needs (%)		
	Pudding (80g)	Muffin (38g)	Cookies (20g)			Pudding (80g)	Muffin (38g)	Cookies (20g)
Energy (kcal)	26	84	95	1,700	170–255	6.54–9.81	2.02–3.04	1.79–2.68
Carbohydrate (%)	4.61	9.98	13.89	275	27.50–41.25	5.97–8.95	2.76–4.13	1.98–2.97
Fat (%)	0.51	3.89	3.93	55.5	5.50–8.25	10.78–16.18	1.41–2.12	1.40–2.10
Protein (%)	0.83	2.46	0.98	3.5	3.60–5.40	4.34–6.51	1.46–2.20	3.67–5.51
Dietary fiber (%)	3.16	2.70	1.91	25.0	2.50–3.75	0.79–1.19	0.93–1.39	1.31–1.96
Resistant starch (%)	0.65	1.36	1.20	-	-	-	-	-

decrease in blood glucose in diabetics can occur by eating foods containing 1.4–48 g of resistant starch daily. Other studies have also shown that consumption of 2 g resistant starch for 4 weeks in diabetic rats affected the postprandial insulin repair and glucose response (Zhou *et al*. 2015).

Fiber intake can maintain the satiety level that will lead to a decrease in calorie intake, beneficial for weight loss as well as improving insulin resistance. The fiber mechanism in glucose metabolism is related to the function and characteristics of fibers. Water-soluble fiber can absorb fluids and form gels in the stomach. Gel slows down the gastric-emptying process and absorption of nutrients. Gel can slow the peristalsis of nutrients (glucose) from the small intestine to the absorption area so that it can stabilize the blood glucose levels (Weickert & Pfeiffer 2018).

In addition, the undigested part of the fiber will go into the large intestine and will be converted into a substrate that can be fermented by bacteria. Fiber fermentation by bacteria produces short-chain fatty acids such as acetate, propionate, and butyrate. These fatty acids will be absorbed back into the bloodstream. Acetate may reduce free fatty acids in the bloodstream for a long time. Propionate can inhibit HMG-CoA reductase, inhibit fat mobilization, prevent the process of gluconeogenesis in the liver, and reduce the reduction of free fatty acids in the blood. This will improve the blood glucose levels and insulin sensitivity (Todesco *et al*. 1991; Luo *et al*. 2000).

Consumption of food with high resistant starch content is the most effective prevention to maintain normal blood glucose concentration and prevent secondary complications resulting from sustained hyperglycemia (Koh & Rowling 2017). Resistant starch can also increase satiety because it can increase genetic expression stimulating satiety associated with GLP-1 and PYY hormones in the large intestine (Okoniewska & Witwer 2007). Resistant starch also functions in increasing insulin sensitivity and reducing abdominal adiposity in obese diabetics (Koh & Rowling 2017).

CONCLUSION

Based on the hedonic test, the pudding and muffin formulas selected were those with a ratio of sago starch to tempeh of 1:1, and for the cookies the selected formula was that with the ratio of sago starch to tempeh of 2:1. Puddings are categorized as a fiber-source food with a negligible level of resistant starch, muffins as a high-fiber food with an intermediate level of resistant starch, and cookies as a high-fiber food with a high level of resistant starch. In conclusion, these products had fullfilled the expectation that they are able to help control diabetics' blood glucose. Further research related to preclinical testing of puddings, muffins, and cookies needs to be conducted to determine the positive role of the products in controlling the blood glucose levels in diabetics.

ACKNOWLEDGEMENT

The author would like to thank the Jambi Provincial Government which has funded this research through the 2017 Achievement Scholarship program. The authors also thank Syartiwidya for providing sago starch as the raw material for making the product in this study. The authors have no conflict of interest.

REFERENCES

[AACC] American Association of Cereal Chemist. 2001. The definition of dietary fiber: Report of the dietary fiber definition committee to the board of directors of the American association of cereal chemist. Cereal Foods World 46(3):112–126.

[AOAC] Association of Official Analytical Chemists. 1995. Official Method of Analysis of Association of Official Analytical Chemist. Washington DC: AOAC International.

Ahmad FB, Williams PA. 1998. Rheological properties of sago starch. J Agric Food Chem 46(10):4060–4065. https://doi.org/10.1021/jf980381o.

Ahmad FB, Williams PA, Doublier JL, Durand S, Buleon A. 1999. Physico-chemical characterisation of sago starch. Carbohydr Polym 38(4):361–370. https://doi.org/10.1016/S0144-8617(98)00123-4 .

Ajila, CM, Leelavathi K, Rao UJSP. 2008. Improvement of dietary fibre content and antioxidant properties in soft dough biscuits with the incorporation of mango

peel flour. J Cereal Sci 48(2):319–326. https://doi.org/10.1016/j.jcs.2007.10.001.
Almatsier S. 2010. Penuntun Diet. Jakarta: Gramedia Pustaka Utama.
Ashfiyah VN. 2019. Substitusi sorgum dan ubi jalar putih pada roti bagel sebagai alternatif selingan untuk penderita diabetes. Media Gizi Indonesia 14(1):75–86. http://dx.doi.org/10.20473/mgi.v14i1.75-86.
[BSN] Badan Standardiasasi Nasional. 2006. SNI Uji makanan [kornet sapi] 01-3775-2006. https://kupdf.net/download/14693-sni-01-3775-2006-kornet-daging-sapi_58f6d9a9dc0d608453da980a_pdf. [Accessed 27th November 2018].
Budzaki S, Komlenic DK, Cacic JK, Cacic F, Jukic M, Kozul Z. 2014. Influence of cookies composition on temperature profiles and qualitative parameters during baking. Croat J Food Sci Technol 6(2):72–78. https://hrcak.srce.hr/131736.
Claudia EJ, Widjanarko SB. 2016. Studi daya cerna (in vitro) biskuit tepung ubi jalar kuning dan tepung jagung germinasi. Jurnal Pangan dan Agroindustri 4(1):391–399.
Cronin K, Preis C. 2000. A statistical analysis of biscuit physical properties as affected by baking. J Food Eng 46(4): 217–225. https://doi.org/10.1016/S0260-8774(00)00053-4.
Darmawan M, Peranginangin R, Syarief R, Kusumaningrum I, Fransiska D. 2014. Pengaruh penambahan karaginan untuk formulasi tepung puding instan. JPB Perikanan 9(1):83–95. http://dx.doi.org/10.15578/jpbkp.v9i1.102.
Deepa DV, Kiran BR, Gadwalkar SR. 2014. Macrovascular and microvascular complications in newly diagnosed type 2 diabetes mellitus. IJCP 25(7):644–648.
[DHHS] Department of Health and Human Services, [USDA] United States Department of Agriculture. 2015. Dietary guidelines for Americans 2015-2020. https://health.gov/dietaryguidelines/2015/guidelines/appendix-13/ [Accessed 12th October 2018].
Ghozali DS, Handharyani E, Rimbawan. 2010. Pengaruh tempe terhadap kadar gula darah dan kesembuhan luka pada tikus diabetik. Jurnal Cermin Dunia Kedokteran 37(3):166–173.
Goni I, Garcia-Diz L, Manas E, Saura-Calixto F. 1996. Analysis of resistant starch: A method for foods and food products. Food Chem 56(4):445–449. https://doi.org/10.1016/0308-8146(95)00222-7.
Goswami D, Gupta RK, Mridula D, Sharma M, Tyagi SK. 2015. Barnyard millet based muffin: Physical, textural, and sensory properties. LWT Food Sci Technol 64(1): 374-380. https://doi.org/10.1016/j.lwt.2015.05.060.
Gourineni V, Stewart ML, Skorge R, Sekula BC. 2017. Slowly digestibility carbohydrate for balanced energy: In vitro and in vivo evidence. Nutrients 9(11):1–10. https://doi.org/10.3390/nu9111230.
Hernández-Ochoa L, Aguirre-Prieto YB, Nevárez-Moorillon GV, Gutierrez-Mendez N, Salas-Munoz E. 2011. Use of essential oils and extracts from spices in meat protection. JFST 51(5):957–963. https://doi.org/10.1007/s13197-011-0598-3.
Horstmann SW, Belz MCE, Heitmann M, Zannini E, Arendt EK. 2016. Fundamental study on the impact of gluten-free starches on the quality of gluten-free model breads. Foods 5(2):1–12. https://doi.org/10.3390/foods5020030.
[Kementan] Kementerian Pertanian. 2012. Muffin sagu keju. http://pusat-pkkp.bkp.pertanian. go.id/ [Accessed 21th March 2018].
Liu KS. 1997. Soybeans: Chemistry, technology, and utilization. New York: Chapman & Hall.
Lockyer S, Nugent AP. 2016. Health effects of resistant starch. Nutr Bull 42(1):10–41. https://doi.org/10.1111/nbu.12244.
Luo J, Yperselle MV, Rizkalla SW, Rossi F, Bornet FR, Slama G. 2000. Chronic consumption of short chain fructooligosaccharides does not affect basal hepatic glucose production or insulin resistance in type 2 diabetics. J Nutr 130(6):1572–1577. https://doi.org/10.1093/jn/130.6.1572
Koh GY, Rowling MJ. 2017. Resistant starch as a novel dietary strategy to maintain kidney health in diabetes mellitus. Nutr Rev 75(5):350–360. https://doi.org/10.1093/nutrit/nux006.
Mirmiran P, Bahadoran Z, Azizi F. 2014. Functional foods-based diet as a novel

dietary approach for management of type 2 diabetes and its complications: A review. World J Diabetes 5(3):267–281. doi: 10.4239/wjd.v5.i3.267.

Norhidayah M, Noorlaila A, Izzati NFA. 2014. Textural and sensorial properties of cookies prepared by partial substitution of wheat flour with unripe banana (*Musa x* paradisiaca var. Tanduk and *Musa acuminata* var. Emas) flour. Int Food Res J 21(6):2133–2139.

Okoniewska M, Witwer RS. 2007. Natural resistant starch: an overview of health properties a useful replacement for flour, resistant starch may also boost insulin sensitivity and satiety. New York: Nutritional Outlook.

[PERKENI] Perkumpulan Endokrinologi Indonesia. 2015. Konsensus Pengelolaan dan Pencegahan Diabetes Melitus tipe 2 di Indonesia. Jakarta: PB. Perkeni.

Peryam DR, Pilgrim PJ. 1957. Hedonic scale method for measuring food preferences. Food Technol 11:9–14.

Poetker. 2010. Eating with diabetes: Desserts and sweets. https://www.sparkpeople.com/resource/nutrition_articles.asp?id=1583. [Accessed 12th October 2018].

Post RE, Mainous AG, King DE, Simpson KN. 2012. Dietary fiber for the treatment of type 2 diabetes mellitus: A meta-analysis. J Am Board Fam Med 25(1):16–23. https://doi.org/10.3122/jabfm.2012.01.110148.

Purwani EY. 2011. Penghambatan proliferasi sel kanker kolon HCT-116 oleh produk fermentasi pati resisten tipe 3 sagu dan beras [Thesis]. Bogor: Institut Pertanian Bogor.

Rismaya R. 2016. Pengaruh substitusi tepung labu kuning (*Curcubita moschata* D.) terhadap sifat fisikokimia, sensori dan kadar serat pangan muffin [Thesis]. Bogor: Institut Pertanian Bogor.

Rudkowska I. 2009. Functional foods for health: Focus on diabetes. Maturitas 62(3):263–269. https://doi.org/10.1016/j.maturitas.2009.01.011.

Saputri I, Damayanti E. 2015. Penambahan pegagan (*Centella Asiatica*) dengan berbagai konsentrasi dan pengaruhnya terhadap sifat fisiko-kimia cookies sagu. J Gizi Pangan 10(2):149–156. https://doi.org/10.25182/jgp.2015.10.2.%25p.

Struck S, Gundel L, Zahn S, Rohm H. 2016. Fiber enriched reduced sugar muffins made from iso-viscous batters. LWT Food Sci Technol 65:32–38. https://doi.org/10.1016/j.lwt.2015.07.053.

Todesco T, Rao AV, Bosello O, Jenkins DJ. 1991. Propionate lowers blood glucose and alters lipid metabolism. Am J Clin Nutr 54(5):860–865. https://doi.org/10.1093/ajcn/54.5.860

Trckova J, Stetina, J, Kansky J. 2004. Influence of protein concentration on rheological properties of carrageenan gels in milk. Int Dairy J 14(4):337–343. https://doi.org/10.1016/j.idairyj.2003.10.004.

Wahjuningsih SB, Marsono Y, Praseptiangga D, Haryanto B. 2016. Resistant starch content and glycaemic index of sago (*Metroxylon spp.*) starch and red bean (*Phaseolus vulgaris*) based analougue ric. Pak J Nutr 15(7):667–672. doi:10.3923/pjn.2016.667.672.

Weickert MO, Pfeiffer AFH. 2018. Impact of dietary fiber consumption on insulin resistance and the prevention of type 2 diabetes. J Nutr 148(1):7–12. https://doi.org/10.1093/jn/nxx008.

Widiawati AA, Anjani G. 2017. Cookies tepung beras hitam dan kedelai hitam sebagai alternatif makanan selingan indeks glikemik rendah. Journal of Nutrition College 6(2):128–137. https://doi.org/10.14710/jnc.v6i2.16902.

World Food and Wine. 2005. Describing foods. https://world-food-and-wine.com/describing-food [Accessed 27th November 2018].

Wrigley C, Békés F, Bushuk W. 2006. Gluten: A balance of gliadin and glutenin. USA: AACC International.

Zhou Z, Wang F, Ren X, Wang Y, Blanchard C. 2015. Resistant starch manipulated hyperglycemia/hyperlipidemia and related genes expression in diabetic rats. Int J Biol Macromol 75:316–321. https://doi.org/10.1016/j.ijbiomac.2015.01.052.

Available online: http://journal.ipb.ac.id/index.php/jgizipangan
Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019
ISSN 1978-1059 EISSN 2407-0920

J. Gizi Pangan, March 2020, 15(1):37-44
DOI: 10.25182/jgp.2020.15.1.37-44

Golden Berry (*Physalis peruviana*) Juice for Reduction of Blood Glucose and Ameliorate of Insuline Resistance in Diabetes Rats

Larasati Sekar Kinasih[1*], Kis Djamiatun[2], Ahmad Ni'matullah Al-Baarri[3]
[1]Nutrition Science Study Program, Faculty of Medicine, Diponegoro University, Semarang 50244, Indonesia
[2]Biomedical Science Department, Faculty of Medicine, Diponegoro University, Semarang 50244, Indonesia
[3]Food Technology Department, Faculty of Animal and Agricultural Science, Diponegoro University, Semarang 50244, Indonesia

ABSTRACT

The study aimed to gather evidence on the potential of golden berry (GB) juice in improving blood glucose level, insulin level, and insulin resistance in type-2 diabetes mellitus (T2DM) in comparison to quercetin supplement in animal model. This study used true experimental pre-post-test study with control group design. Twenty five Wistar male rats were divided into five groups: healthy group (K-), T2DM positive control group (K+), T2DM group with 1 ml/200 g BW/day of GB juice (X1), T2DM group with 5 ml/200 g BW/day of GB juice (X2), and T2DM group with 6 mg/200 g BW/day of quercetin (X3). The T2DM rasts were obtained from healthy rats induced by high-fat feed and Streptozotocin (STZ). The result showed that various dosages of GB juice (X1 and X2) were able to lower blood glucose level (-79.15; -110.44; -108.20) and HOMA-IR (-2.40; -2.92; -3.02). In addition, it was also able to increase insulin level (0.26; 1.99; 1.42) compared to (K+) group ($p<0.05$). In conclusion, GB juice was able to lower blood glucose level, insulin resistance, and increase insulin level in T2DM rats. The GB juice dosage of 1 ml/200 g BW/day and 5 ml/200 g BW/day were better in lowering the blood glucose level and improving insulin resistance compared to quercetin supplement.

Keywords: blood glucose, golden berry juice, insulin resistance, type-2 diabetes mellitus

INTRODUCTION

Type-2 diabetes mellitus (T2DM) is a metabolic disorder marked by elevated blood sugar levels (hyperglycemia) due to decrease in pancreatic β-cell function or insulin resistance (Kahn *et al*. 2014). Type-2 diabetes mellitus, is a global pandemic. International Diabetes Federation (IDF) stated that in 2017, globally there was 425 million cases of T2DM and has been expected to reach 629 million cases in 2045. In Indonesia alone, the number of T2DM was 10 million cases in 2015 (International Diabetes Federation 2017).

One of the underlying pathophysiology of T2DM is insulin resistance (Kahn *et al*. 2014), it is a complex metabolic disorder where the tissue capabilities to use insulin is reduced. Continous high intake of calories and accumulation of free fatty acids in some tissues can lead to insulin resistance, which perturb the utilization of glucose in the tissues (liver, muscle, and fat tissue). The insulin resistance causes reduction of insulin interaction with tissues and further disrupts the metabolic pathways of glucose, fat, and protein (Sah *et al*. 2016).

The hyperglycemia in type-2 diabetes mellitus increases the formation of advanced glycation end products (AGEs) and glucose oxidation which produce reactive oxygen species (ROS). The presence of ROS further plays a role in β-cell damage and reduces insulin sensitivity (Qatanani & Lazar 2007). The imbalance of free radicals and antioxidants in the body causes oxidative stress in T2DM (Chikezie *et al*. 2015). Many studies have found that flavonoid content in food has a major role as antioxidant to improve T2DM (Anhe *et al*. 2013). The golden berry (*Physalis peruviana*) is one of such food that is also commonly utilized in Indonesia. The phenolic content in golden berry was 50–250 mg/100 g of fruit and its antioxidant activity was

[*]**Corresponding Author:** tel: +628563588384, email: larasatisekark@gmail.com

210.82 μmoltrolox/100 g of fruit (Puente *et al*. 2011). Research also shown that golden berry has a role as anti-inflammatory, antioxidant, and anti-diabetic (Sathyadevi *et al*. 2014; Puente *et al*. 2011). The main phenolic compounds in GB fruit are quercetin, myrecetin, and kaempferol (Puente *et al*. 2011).

Fruit juice is considered as functional food because it still contain the micronutrients or active substances from fresh fruits that provides physiological advantages (Ramadan & Moersel 2006). Hassan & Ghoneim (2013) stated that the administration of GB juice with a 1 ml/200 g BW/day dose in diabetic rats, could lower blood sugar significantly compared to untreated diabetic group. Quercetin content on golden berry extract in this study was able to improve pancreas β-cells function as an antioxidant agent. In recent years, quercetin was not only available in food form but also in supplementation and generally introduced as therapy for bacterial infections, gout, hypertension, diabetes mellitus, asthma, and many more (Larson *et al*. 2012). Despite the ease of use of supplement, some patient prefer natural fresh remedies such as the golden berry fruit juice for long term use. Therefore, this study aimed to find evidences on the potential of the golden berry juice compared to quercetin supplement in improving blood glucose level, insulin level, and insulin resistance in type-2 diabetes mellitus rats. The results is expected to contribute to further study for Golden Berry functional food product development and efficacy testing in human subject.

METHODS

Design, location, and time

The study used true experimental study with completely randomized design pre-post-test with control group design. This study was conducted at the Integrated Laboratory of Diponegoro University, Semarang for testing the content of quercetin in golden berry juice. Animal care and testing were conducted at Center for Food and Nutrition Research Laboratory, Gadjah Mada University, Yogyakarta from December 2017 to February 2018.

Sampling

Determination of sample size in this study was based on World Health Organization (WHO 2000) minimum sample for animal assay, which is 5 rats for each treatment group (WHO 2000). Twenty five male Wistar aged 8–12 weeks with 150–200 g of body weight were obtained from Center for Food and Nutrition Research Laboratory, Gadjah Mada University, Yogyakarta. GB fruits were obtained from the garden in Ciwidey, Bandung regency. Quercetin was obtained from Sigma Aldirch (Q4951, St.Louis, USA), Streptozotocin (STZ) was obtained from Nacalai Tesque Japan and Niconitamide (NA) was obtained from Nacalai Tesque, Japan. This study also used blood glucose check reagent (Dyasis) and insulin ELISA kit (Fine Test).

The standard rat feed was consisted of Confeed AD II (composition: 12% water, 15% crude protein, 3–7% crude lipid, 6% crude fiber, 7% ash, 0.9–1.1% calcium, and 0.6–0.9% phosphorus) and the high-fat feed (composition: 80% standard feed, 20% lard, and 1% extra cholesterol) were obtained from Center for Food and Nutrition Research Laboratory, Gadjah Mada University, Yogyakarta. Tools for animal care like cages, feeding plate, drinking bottle, digital scales, and blood sampling tools used micro hematocrit and syringe probe.

GB juices were made using basins, blender, cheese cloth, spoon, and measuring cup. Blood analysis used a blood glucose check and insulin ELISA kit. Tools and materials used for testing the content of quercetin in GB juice were 70% methanol, distilled water, Erlenmeyer tubes, beaker glasses, pipettes, ultrasonic (Branson), Whatman paper, micropipette 1,000 μL and 10-100 μL, test tubes, and High Performance Liquid Chromatography (HPLC) (Shimadzu, Japan).

Procedures

Testing for Quercetin content in golden berry juice. Five ml of the sample juice were mixed with 25 ml of 70% methanol and extracted using the ultrasonic at 30^0C for 20 minutes. Extracted sample was stored in freezing temperature for 24 hours and then filtered through a 0.45 μm filter. A 20 ml of sample was induced to HPLC instrument. The HPLC analysis was performed using a Shimadzu-LC system equipped with a UV-Vis detector (SPD-20AV), a column Purospher® STAR C18 (250 mm x 4.0 mm, 5 μm), a flow rate of 1.1 ml/minutes, a column temperature of 30^0C, a mobile phase of Methanol : Acetonitrile : Water (60:20:20) % v/v and a detection wavelenght of 254 nm.

Conditioning of type-2 diabetes mellitus. Twenty-five Wistar male rats were acclimatized to individual cages for 7 days given the standard feed and water. The room temperature ranged 25–28^{0}C with 12 hours light cycle (6:00 a.m. to 6:00 p.m.). Rat's cages was cleaned every day. Blood sampling was conducted through plexus retroorbitalis to standardize blood glucose before it was conditioned with type-2 diabetes mellitus. Twenty rats were conditioned T2DM with high-fat feeding for 14 days (Sathyadevi *et al*. 2014) followed by NA induction with the dose of 110 mg/kg body weight via the intraperitoneal injection and 15 minutes later were given the STZ intraperitoneal induction with a dose of 45 mg/kg body weight (Ghasemi *et al.* 2014). Before the STZ induction, rats were fasted for 8–10 hours. After 3 days of STZ induction, rats were fasted for 8–10 hours (Gheibi *et al*. 2017) and 2 ml blood drawn through the plexus retroorbitalis for blood glucose and insulin levels analysis. Rats full filed the T2DM criteria if the fasting serum glucose levels was ≥200 mg/ (Gheibi *et al*. 2017).

Treatments of golden berry juice and quercetin. Rats were divided into 5 groups: healthy group without treatments (K-), T2DM positive control group without treatments (K+), T2DM group with treatment of 1 ml/200 g BW/day of GB juice (X1), T2DM group with treatment of 5 ml/200 g BW/day of GB juice (X2), and T2DM group with treatment of 6 mg/200 g BW/day of quercetin (X3). The dose of 1 ml/200 g BW/day of GB juice was based on Hassan & Ghoneim (2013) who stated that the dose could lower blood glucose in diabetic rats significantly, while the 5 ml/200 g BW/day dose of GB juice was based on ±1 glass of fruit juice consumed by humans. Quercetin was used as standard therapy in experimental animals with T2DM. The 6 mg/200 g BW/day dose of GB juice was the effective dose to lower blood glucose significantly compared to T2DM control group based on previous study of Chis *et al*. (2015).

Quercetin was homogenized with a 0.5% Sodium-Carboxymethil cellulose (NA-CMC) each into 5 ml of volume. The making of GB juices and quercetin solution were prepared every day in the morning then immediately administered to T2DM rats via a gastric tube once a day. The GB juice and quercetin in T2DM rats were administered for 28 days. Blood sampling was conducted at the end of the study through plexus retroorbitalis for analysis of blood glucose and insulin levels. Insulin resistance level was indicated by the value of Homeostasis Model Assessment Insulin Resistance (HOMA-IR) with the formula as follow; HOMA-IR = (blood glucose level (mg/dl) x insulin level (µU/ml) divided by 405 (Esteghamati A *et al*. 2010). The study methods had received approval from the Ethics Committee for Health Research in Diponegoro University and Dr. Kariadi Hospital, register number 89/EC/H/FK-RSDK/XII/2017.

Data analysis

Data normality was tested by Shapiro-Wilk test. All of the data had normal distributions, thus the testing for the differences between before and after treatments used the paired t-test. Analysis of the effects of the differences between groups used the One-Way ANOVA test followed Tamhane post hoc ($p<0.05$) for blood gluce and HOMA-IR analysis, while for insulin analysis it was followed by Bonferroni post hoc test ($p<0.05$).

RESULTS AND DISCUSSION

The quercetin content in GB juices and fruit form have been reported previously (Puente *et al*. 2011). The quercetin's content of GB juice in this study was 20.775 µg/ml of juice.

Conditioning of type-2 diabetes mellitus. Prior to the treatment of GB juice and quercetin, blood glucose level was tested as a standard category of healthy rats and showed the homogeneous level of blood glucose level in the samples (Table 1). Conditioning of type-2 diabetes mellitus in this study was conducted by high-fat feeding for 14 days and induction of STZ. Blood glucose levels of all rats were more than 200 mg/dl after conditioning of T2DM (Table 2).

High-fat feeding aimed to establish condition of insulin resistance. It led to reduction in the activation of phosphatidylinositol 3-kinase (PI3K)/Akt, which affected tyrosine phosphorylation and decreased IRS so it could reduce insulin signaling (Gheibi *et al*. 2017). The STZ inducted later causes a breakdown of deoxyribonucleic acid strand (DNA) and overexpression activation of poly synthase (ADP-ribose) which was an enzyme to repair DNA. The rats was induced with NA at 15 minutes prior the STZ, it aimed to provide protection for pancreatic

Table 1. The initial glucose level standardization

Treatment groups	Initial glucose levels	p
K(-)	73.94±15.76	
K(+)	69.26±5.77	
X1	87.46±9.06	0.319
X2	73.27±5.93	
X3	69.26±10.59	

p; One-Way ANOVA test. K(-): healthy group + without treatments, K(+): T2DM + without treatments; X1: T2DM group + GB Juice 1 ml/200 g BW/day (X1); X2: T2DM group + GB Juice 5 ml/200 g BW/day; X3: T2DM group + Quercetin 6 mg/200 g BW/day; n: 25 samples

β-cells from toxic effects of excessive STZ and created type-2 diabetes mellitus in rats (Zuloaga *et al*. 2014)

The administration of golden berry juice on levels of blood glucose, insulin, and HOMA-IR. Paired t-test showed that there were a decrease in blood glucose level (Table 2), an increase in insulin level (Table 3), and a decrease in HOMA-IR value (Table 4) with treatment of GB juice with a 1 ml/200 g BW/day dose and 5 ml/200 g BW/day dose significantly in the before and after treatments ($p<0.05$). There were significant differences in changes of mean value with GB juice X1 and X2 treatments compared to the K(+) in lowering blood glucose level, increasing insulin level, and decreasing HOMA-IR ($p<0.05$). This findings were in line with Hassan & Ghoneim (2013) who stated that administration of GB juice 1 ml/day could lower blood glucose significantly compared to diabetic control group and blood glucose level in group with The GB juice treatment was comparable to healthy rats group. Further, Sathyadevi *et al.* (2014) also found that GB extract could lower blood glucose and increase insulin levels significantly compared to diabetic control group.

The decreased values of blood glucose level in treatments of GB juice 1 ml/200 g BW/day and 5 ml/200 g BW/day were equal to quercetin at dose of 6 mg/200 g BW/day. It was no significant differences between treatments in X1, X2, and X3 in the Tamhane post hoc test ($p>0.05$) (Table 3). The largest decrease in blood glucose level was shown by the GB juice tearment of 5 ml/200 g BW/day with the value of 110.44 ± 10.63. It was most likely influenced by the rich antioxidants contents of GB juice in addition to its quercetin content. Previous study found that GB juice contains several phenolic substance, including quercetin as major phenolic content and followed by myrecetin and kaempferol (Puente *et al*. 2011) which could play a role in decreasing blood glucose level.

As anti-diabetic agents, quercetin, myrecetin, and kaempferol available at various tissues, such as in the muscle. Quercetin for example, promotes glucose uptake in the skeletal muscle tissue by increasing the glucose transporter 4 (GLUT4) translocation. In the liver, these agents

Table 2. Blood glucose levels before and after treatments of golden berry juices and quercetin

Treatment groups	Blood glucose level (mg/dl)		Δ	p'	p
	Before	After			
K(-)	82.76±6.17	100.93±4.50	18.17±6.16[a]	0.003*	
K(+)	212.93±9.42	282.30±20.75	69.37±25.31[a]	0.004*	
X1	221.02±9.97	141.87±6.05	-79.15±12.56[ab]	0.000	0.000*
X2	224.16±11.91	113.72±4.43	-110.44±10.63[ab]	0.000*	
X3	214.05±14.04	105.85±2.51	-108.20±14.12[a]	0.000*	

p': Paired t-test; p: One-Way ANOVA test; [a] $p<0.05$ Tamhane post hoc test with K(+); [b] $p<0.05$ Tamhane post hoc test with X1; no significant differences in X2 and X3; K(-): healthy group + without treatments; K(+): T2DM + without treatments; X1: T2DM group + GB Juice 1 ml/200 g BW/day (X1); X2: T2DM group + GB Juice 5 ml/200 g BW/day; X3: T2DM group + Quercetin 6 mg/200 g BW/day; n: 25 samples

Table 3. Mean values of insulin level before and after treatments of GB juices and quercetin

Treatment groups	Blood glucose level (mg/dl)		Δ	p'	p
	Before	After			
K(-)	16.49±0.19	15.65±0.22	-1.09±0.39[abc]	0.003*	
K(+)	12.72±0.16	11.82±0.14	-0.90±0.19[bc]	0.000*	
X1	12.76±0.12	13.02±0.14	0.26±0.11[ac]	0.007*	0.000*
X2	12.74±0.26	14.53±0.17	1.99±0.30[ab]	0.000*	
X3	12.69±0.23	14.12±0.18	1.42±0.35[abc]	0.001*	

p': Paired t test; p: One-Way ANOVA test; [a] p<0.05 Bonferroni post hoc test with K(+); [b] p<0.05 Bonferroni post hoc test with X1; [c] p<0.05 Bonferroni post hoc test with X2; K(-): healthy group + without treatments; K(+): T2DM + without treatments; X1: T2DM group + GB Juice 1 ml/200 g BW/day (X1); X2: T2DM group + GB Juice 5 ml/200 g BW/day; X3: T2DM group + Quercetin 6 mg/200 g BW/day; n: 25 samples

improve the glucokinase activity to increase glucose storage in the liver through activation of AMP-activated protein kinase (AMPK). AMPK is an enzyme that regulates the homeostatic energy in the body through several mechanisms such as inhibiting the gluconeogenesis, increases the fatty acid oxidation, and increases the expression of GLUT4. In the gut, these agents help to decrease maltase activity and glucose transporter 2 (GLUT2) that can reduce the absorption of glucose in the gut (Anjani *et al*. 2018; Alkhalidy *et al*. 2015).

The decreased insulin levels indicated a functional disorder of pancreatic β-cells and a reduction in pancreatic islet cells because conditioning of type-2 diabetes mellitus by high-fat feeding and induction of STZ. Antony *et al*. (2017) stated that histopathology test of pancreatic tissue in animal fed by high-fat and STZ induction showed reduction in pancreatic islet cells. This condition could form amyloid which triggered the ROS. The changes in insulin levels were significantly different between X1, X2, and X3 in Bonferroni post hoc test (p<0.05) (Table 4). The highest elevated levels of insulin could be seen in treatment of GB juice of 5 ml/200 g BW/day with a mean value of 1.99±0.30. It was thought because of other active substances contents in addition to quercetin that help protect pancreatic β-cell and worked as an antioxidant

Table 4. Mean values of HOMA-IR before and after treatments of GB juices and quercetin

Treatment groups	Blood glucose level (mg/dl)		Δ	p'	p
	Before	After			
K(-)	3.42±0.28	3.89±0.13	0.48±0.22	0.028*	
K(+)	6.69±0.29	8.24±0.61	1.55±0.82	0.027*	
X1	6.96±0.33	4.56±0.17	-2.40±0.39[a]	0.000*	0.000*
X2	7.06±0.40	4.14±0.19	-2.92±0.29[a]	0.000*	
X3	6.71±0.34	3.68±0.09	-3.02±0.37[a]	0.000*	

p': Paired t test; p: One-Way ANOVA test; [a] p<0.05 Tamhane post hoc test with K(+); K(-): healthy group without treatments; K(+): T2DM + without treatments; X1: T2DM group + GB Juice 1 ml/200 g BW/day (X1); X2: T2DM group + GB Juice 5 mL/200 g BW/day; X3: T2DM group + Quercetin 6 mg/200 g BW/day; n: 25 samples

agents. Puente *et al.* (2011) stated that GB juice contained vitamin C, vitamin E, quercetin, myericetin, and kaempferol. Those contents can help quercetin in GB juice in providing more protection to β-cells as antioxidant agents. (Garcia-Bailo *et al*. 2011; Al-Numair *et al*. 2015) compared to quercetin alone.

GB juice with a dose of 1 ml/200 g BW/ day and 5 ml/200 g BW/day had same effect with a 6 mg/200 g BW/day of quercetin treatment in improving insulin resistance as shown in Tamhane post hoc test with p>0.05 at X1, X2 and X3 treatments (Table 4). Previous study found that phenolic compounds and antioxidant activity in GB juice was high. Phenolic compound in GB juice provides a great potential for prevention or management of chronic disease.

Our findings supports the Choi *et al*. (2015) findings which stated that administration of quercetin for 10 weeks to rats induced by STZ could lower HOMA-IR significantly compared to diabetic control group (Choi *et al*. 2015). Quercetin is a powerful antioxidant that can capture free radicals and bind transition metal ions. Quercetin is also one of the best ROS (O2- and ONOO-) catcher. Anti-inflammatory effect of quercetin was able to reduce levels of pro-inflammatory cytokines like tumor necrosis factor alpha (TNF-α) by inhibiting the expression of Nuclear Factor Kappa Beta (NF-Kβ) (Choi *et al*. 2015; Luo *et al*. 2015). The inhibition of NF-Kβ expression could reduce insulin receptor substrate (IRS)-1 serine phosphorylation and increase expression of IRS-1 (Luo *et al*. 2015). IRS-1 protein was an important protein in improving insulin sensitivity.

CONCLUSION

We found evidences that GB juice was able to lower blood glucose level, increase insulin level, and improve insulin resistance. The effect of GB juice with a dose of 1 ml and 5 ml is comparable to quercetin with an adimistration dose of 6 mg/200 g BW/day in improving the blood glucose and HOMA-IR. In addition, the GB juice was better than quercetin in improving the insuline level. This corroborated the findings that GB juice is a potential nutrition support for type-2 diabetes mellitus. Further research is necessary to test the complete phytochemical contents of GB juice and to analyze its effect on other anti-inflammatory and antioxidant parameters in T2DM to deepen our understanding on the role and mechanisms of GB juice in T2DM.

ACKNOWLEDGEMENT

The author would like to thank to Integrated Laboratory of Diponegoro University, Semarang, Center for Food and Nutrition Research Laboratory, Gadjah Mada University, Yogyakarta, Diponegoro University for access and technical support. The authors have no conflict of interest.

REFERENCES

Alkhalidy H, Moore W, Zhang Y, McMillan R, Wang A, Ali M, Suh KS, Zhen W, Cheng Z, Jia Z *et al*. 2015. Small molecule kaempferol promotes insulin sensitivity and preserved pancreatic *β*-cell mass in middle-aged obese diabetic mice. J Diabetes Res 20015:1–14. http://dx.doi.org/10.1155/2015/532984.

Al-numair KS, Chandramohan G, Veeramani C, Alsaif MA. 2015. Ameliorative effect of kaempferol, a flavonoid, on oxidative stress in streptozotocin-induced diabetic rats. Redox Rep 20(5):198–209. https://doi.org/10.1179/1351000214Y.0000000117.

Anhe FF, Desjardins Y, Pilon G, Dudonne S, Genovese MI, Lajolo FM, Marette A. 2013. Polyphenols and type 2 diabetes : A prospective review. Pharma Nutrition 1(4):105–114. http://dx.doi.org/10.1016/j.phanu.201307.004.

Anjani PP, Damayanthi E, Rimbawan R, Handharyani, E. 2018. Potential of okra (*Abelmoschus esculentus* L.) extract to reduce blood glucose and malondialdehyde (MDA) liver in streptozotocin-induced diabetic rats. J Gizi Pangan 13(1):47–54. http://dx.doi.org/10.25182/jgp.2018.13.1.47-54.

Antony PJ, Gandhi GR, Stanlin A, Balakrishna K, Toppo E, Sivasankaran K, Ignacimuthu S, Al-Dhabi NA. 2017. Myoinositol ameliorates high-fat diet and streptozotocin-induced diabetes in rats through promoting insulin receptor signaling. Biomed Pharmacother 88:1098–1113. https://doi.org/10.1016/j.biopha.2017.01.170

Chikezie PC, Ojiako OA, Ogbuji AC. 2015. Oxidative stress in diabetes mellitus. Int J Biol Chem 9(3):92–109. doi: 10.3923/ijbc.2015.92.109.

Chis IC, Coseriu A, Simedrea R, Orors A, Nagy AL, Clichici S. 2015. *In vivo* effects of quercetin in association with moderate exercise training in improving streptozotocin-induced aortic tissue injuries. Molecules 20(12):21770–2178. https://doi.org/10.3390/molecules201219802.

Choi H, Jeong SM, Huh GH, Kim JI. 2015. Quercetin ameliorates insulin sensitivity and liver steatosis partly by increasing adiponectin expression in *ob/ob* mice. Food Sci Biotechnol 24(1):273–279. https://doi.org/10.1007/s10068-015-0036-9.

Esteghamati A, Ashraf H, Khalilzadeh O, Zandieh A, Nakhjavani M, Rashidi A, Haghazali M, Asgari F. 2010. Optimal cut-off of homeostasis model assessment of insulin resistance (HOMA-IR) for the diagnosis of metabolic syndrome : Third national surveillance of risk factors of non- communicable diseases in Iran (SuRFNCD-2007). Nutr Metab 7(26):1–8. https://doi.org/10.1186/1743-7075-7-26.

Garcia-bailo B, El-Sohemy A, Badawi A. 2011. Vitamins D, C, and E in the prevention of type 2 diabetes mellitus: Modulation of inflammation and oxidative stress. Biologics 2011(5):7–19. doi:10.2147/BTT.S14417.

Ghasemi A, Khalfi S, Jedi S. 2014. Streptozotocin-nicotinamide-induced rat model of type 2 diabetes. Hung Acta Physiol 101(4):408–420. https://doi.org/10.1556/APhysiol.101.2014.4.2

Gheibi S, Kashfi K, Ghasemi A. 2017. A practical guide for induction of type-2 diabetes in rat: Incorporating a high-fat diet and streptozotocin. Biomed Pharmacother 95:605–613. http://dx.doi.org/10.1016/j.biopha.2017.08.098.

Hassan AI, Ghoneim MAM. 2013. A possible inhibitory effect of physalis (*Physalis pubescens* L.) on diabetes in male rats. World Appl Sci J 21(5):681–688. doi:10.5829/idosi.wasj.2013.21.5.7220.

[IDF] International Diabetes Federation, 2017. IDF Diabetes Atlas. https://www.idf.org/e-library/epidemiology-research/diabetes-atlas/134-idf-diabetes-atlas-8th-edition.html [Accessed 17th March 2018].

Kahn SE, Cooper ME, Prato SD. 2014. Pathophysiology and treatment of type 2 diabetes: Perspectives on the past, present and future. The Lancet 383(9922):1068–1083. https://doi.org/10.1016/S0140-6736(13)62154-6.

Larson AJ, Symons JD, Jalili T. 2012. Therapeutic potential of quercetin to decrease blood pressure: Review of Efficacy and mechanisms. American Society for Nutrition Adv Nutr 3(1):39–46. https://doi.org/10.3945/an.111.001271

Luo C, Yang H, Tang C, Yao G, Kong L, He H, Zhou Y. 2015. Kaempferol alleviates insulin resistance via hepatic IKK / NF- κB signal in type 2 diabetic rats. Int Immunopharmacol 28(1):744–750. http://dx.doi.org/10.1016/j.intimp.2015.07.018.

Puente LA, Pinto-Munoz CA, Castro ES, Cortes M. 2011. *Physalis peruviana* Linnaeus, the multiple properties of a highly functional fruit: A review. Food Rres Int 44(7):1733–1740. http://dx.doi.org/10.1016/j.foodres.2010.09.034.

Qatanani M, Lazar MA. 2007. Mechanisms of obesity-associated insulin resistance: Many choices on the menu. Genes Dev 21(12):1443–1455. doi:10.1101/gad.1550907.

Ramadan MF, Moersel JT. 2006. Impact of enzymatic treatment on chemical composition, physicochemical properties and radical scavenging activity of goldenberry (*Physalis peruviana* L.) juice. J Sci Food Agric 87(3):452–460. doi:10.1002/jsfa.2728.

Sah SP, Singh B, Choudhary S, Kumar A . 2016. Animal models of insulin resistance: A review. Pharmacol Rep 68(6):1165–1177. https://doi.org/10.1016/j.pharep.2016.07.010.

Sathyadevi M, Suchithra ER, Subramanian S. 2014. *Physalis peruviana* Linn. fruit extract improves insulin sensitivity and ameliorates hyperglycemia in high-fat diet low dose STZ-induced type 2 diabetic rats. J Pharm Res 8(4):625–632.

[WHO] World Health Organization. 2000. General guidelines for methodologies

on research and evaluation of traditional medicine. World Health Organization Geneva. http://apps.who.int/iris/bitstream/10665/66783/1/WHO_EDM_TRM_2000.1.pdf [Accessed 24th November 2017].

Zuloaga KL, Krasnow SM, Zhu X, Zhang W, Jouihan SA, Shangraw RE, Alkayed NJ, Marks DL. 2014. Mechanism of protection by soluble epoxide hydrolase inhibition in type 2 diabetic stroke. Plos One 9(5):1-9. https://doi.org/10.1371/journal.pone.0097529.

Available online: http://journal.ipb.ac.id/index.php/jgizipangan
Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019
ISSN 1978-1059 EISSN 2407-0920

J. Gizi Pangan, March 2020, 15(1):45-52
DOI: 10.25182/jgp.2020.15.1.45-52

In Vitro α-Glucosidase Inhibition and Antioxidant Activity of Mulberry (*Morus Alba* L.) Leaf Ethanolic Extract

Hasim[1*], Widia Ayu Lestari[1], Uci Sugiman[1], Didah Nur Faridah[2,3]
[1]Department of Biochemistry, Faculty of Mathematics and Natural Sciences,
IPB University, Bogor 16680, Indonesia
[2]Department of Food Science and Technology, Faculty of Agricultural Engineering and Technology,
IPB University, Bogor 16680, Indonesia
[3]Southeast Asian Food & Agriculture Science & Technology (SEAFAST) Center,
IPB University, Bogor 16680, Indonesia

ABSTRACT

This present work aimed to investigate the in vitro antioxidant activity and α-glucosidase inhibitory effect of mulberry leaf ethanolic extract. Antioxidant analysis was performed using Thiobarbituric Acid (TBA) assay at concentrations of 125, 200, 500, and 1,000 ppm. The results showed that the optimum incubation time was four days and the extracts could reduce the formation of MDA, i.e. 41.21%, 45.33%, 44.19%, and 36.00%, respectively. This suggests that concentration of 200 ppm was found as the best treatment. In addition, the result showed that ethanolic extract of mulberry leaf also showed inhibition against α-glucosidase with the IC_{50} of 309.82 µg/mL.

Keywords: α-glucosidase, antioxidant, mulberry, thiobarbituric acid

INTRODUCTION

Free radical is an atom or group of atoms that possess one or more unpaired electrons, which make it highly chemically reactive towards electrons in other molecules of human cells (Amrun *et al*. 2007). It causes disruption of important macromolecules such as protein, lipid, carbohydrate, and DNA, which in turn causing physiological disorders such as diabetes mellitus (DM). Nowadays, the prevalence of DM has been growing around the globe, especially type 2 diabetes mellitus (T2DM). Factors contributing to the disease are aging, social-economic problems, lifestyle, lack of physical activities, and obesity (WHO 2015). In addition, glycemia was proposed as another contributor to T2DM (Barclay *et al*. 2008).

A noticeable increase in glucose level after carbohydrate loading (postprandial glycemia) that exceeds the normal level (hyperclycemia postprandial) is deemed as one of the significant factors causing T2DM (Barclay *et al*. 2008). One way to control postprandial glycemia is via inhibition of glucose absorption in the intestine. In this case, inhibiting the action of intestinal enzymes, such as α-glucosidase, that converts carbohydrate into glucose can be a promising attempt (Castellano *et al*. 2013). This inhibitory action against α-glucosidase is meaningful in management of DM.

Mulberry (*Morus alba* L.) has been reported as a source of glucose inhibitors (Efendi *et al*. 2010) and widely used for various diseases such as diabetes, hyper cholesterolemia, and kidney diseases (Huang *et al*. 2013). The beneficial properties of the plant are attributed to its active compounds such as alkaloid, flavonoid, polyphenol, Calcium, Phosphor, Iron, Manganese, vitamins (A, B, C). Numerous reports have been published regarding the physiological effects of mulberry leaf extracts in controlling glucose level after carbohydrate consumption (Chung *et al*. 2013; Jeszka-Skowron *et al*. 2014), while the extract was known as capable of alleviating the absorption of sucrose and maltose in intestine. Intake of mulberry leaf ethanolic and asetone extracts in streptozotocin (STZ)-induced diabetic rats remarkably reduced glucose level, while also increased insulin concentration and antioxidant activity (Jezka-Skowron *et al*. 2014).

A study on antioxidant activity of mulberry twig and root bark extract estimated using DPPH assay showed protective activities on phospho-

[*]**Corresponding Author:** tel: +681319209692, email: hasimdea@yahoo.com

lipids against free radical attacks; the extract was also effective in avoiding biomolecules from oxidative disruption (Chang *et al*. 2011). The anti oxidant activity of mulberry leaves extracted using different solvents (acetone, methanol, aqueous) were also investigated. The research found that the methanol extract exhibited the highest content of phenolic and antioxidant activity (Arabshahi-Delouee & Urooj 2007).

Further, Jeszka-Skowron *et al*. (2014) demonstrated that mulberry leaf extract isolated with ethanol 65% showed the strongest action on alleviating glucose level of experimental animals. In addition, the experiment showed that such glucose level-lowering effect contributed to increace secretion of insulin and antioxidant activity. However, there is no any scientific report on in vitro experiment uncovering the effects of mulberry leaf ethanolic extract on inhibition of α-glucosidase. This present work aimed to investigate the antioxidant activity using TBA (Thiobarbituric Acid) assay, and evaluate the inhibitory properties of mulberry leaf ethanolic extract against α-glucosidase.

METHODS

Design, location, and time

Antioxidant activity was evaluated according to Completely Randomized Design (CRD) consisting of three groups: negative control (distilled water), positive control (α-tocopherol) at concentration of 200 ppm, and mulberry leaf ethanolic extract at various levels (125, 200, 500 and 1,000 ppm). Each experimental unit was carried out at triplicates.

Materials and tools

Mulberry leaves were collected from UKBB, Indonesia. Chemicals used were ethanol 65%. Ethanol 30%, chloroform, ammonia, H2SO4, Dragendorf reagent, Meyer reagent, Wagner reagent, FeCl3, ether, NaOH 10% were used for phytochemical analysis. In terms of MDA analysis and incubation time determination, some chemicals were used, including ethanol 70%, absolute ethanol, linoleic acid 50 mM, buffer phosphate 0.1 M (pH 7), α-tocopherol (vitamin E), TMP (1,1,3,3-tetramethoxypropane) 6 M, TCA (trichloroacetic acid), TBA (thiobarbituric acid), and glacial acetic acid. Chemicals used for α-glucosidase inhibition analysis included DMSO (Dimethyl Sulfoxide), α-glucosidase, p-nitrophenyl-α-D glucopyranoside (p-NPG), natrium bicarbonate (Na_2CO_3), buffer phosphate, bovine serum albumin (BSA), starch, alloxan, and acarbose (Glucobay). All chemicals used are analytical grade.

Experimental instruments for antoxidant activity analysis were oven, electrical blender, shaker, filter paper, spectrophotometer, centrifuge. While, the instruments used for sample preparation and inhibition analyss were oven, rotary evaporator, microplate, microplate reader Bio Rad, digital balance, vortex.

Procedure

Preparation of mulberry leaf powder (Jeszka-Skowron et al. 2009). Mulberry leaves were sorted (young leaf, bright green-dark green in color, no blackspots). The sorted leaves were washed, shredded, and sun-dried for 1 hour. Next, the leaves were re-dried in oven for 6 hours at 60°C, powdered and filtered.

Moisture analysis (AOAC 2012). Moisture content was analyzed using standard method of AOAC (2012). Briefly, mulberry leaf powder (2 g) was dehydrated in oven for 6 hours at 105°C, desiccated for 15 minutes, and weighed.

***Extraction (Jeszka-Skowron et al. 2014,* modified)**. Mulberry leaves were macerated for 24 hours using ethanol 65% at a ratio of 1:10 (leaf powder: solvent). At the first 6 hours, the mixture was shaken in a shaker at 125 rpm, and left. Macerated substance was collected after filtration using filter paper. This maceration condition was replicated 3 times. The solvent was then removed using rotary evaporator, yielding an extract paste. The % extract yield was then determined as follows:

$$\%yield = \frac{extract\ weight}{water - free\ simplicia\ weight} \times 100\%$$

Phytochemical Analysis (Harborne 1987). In this section, determination of alkaloid, tanin, flavonoid,saponin, steroid, and triterpenoid was carried out.

Determination of incubation time using conjugated dienes (Esterbauer 1989). A mixture consisting of 2 ml buffer phosphate (0.1 M, pH 7), 2 ml linoleic acid (50 mM; in ethanol 99.80%), and 1 ml deionized water was prepared and then placed in screw pan bottle. The mixture

was incubated at 400°C, and its absorbance was measured till obtaining the maximum one, with a descending value. Absorption intensity was measured by adding 50 µL of linoleic acid previously incubated with 6 ml of ethanol 75%. The absorption was read at a wavelength of 234 nm, while ethanol 75% was used as blank solution.

Determination of Antioxidant Activity using TBA assay (Kikuzaki & Nakatami 1993). Ethanolic extract of mulberry leaf was made at different concentrations: 125, 250, 500 and 1,000 ppm. The α-tocopherol (200 ppm) was used as positive control, while distilled water was used as negative control. All of these samples were taken 1 ml, added with 2 ml of buffer phosphate (0.1 M, pH 7) and 2 ml of linoleic acid 50 mM in ethanol 99.8%.

Solution was transferred into a dark bottle with a screw cap, and incubated at 400°C for a particular period of time as determined previously. MDA level was measured by TBA method exactly 2 days after incubation. Briefly, one ml of each solution was added with 2 ml of TCA 2% and 2 ml of TBA 1% in a glacial acetic acid 50%. Distilled water was used as blank solution, with a similar procedure. The mixture was heated at 100°C for 10 minutes, cooled, and centrifuged at 3,000 rpm for 15 minutes. The absorbance was read at 532 nm wavelength using spectrophotometer.

Furthermore, standard curve was made. TMP solution was prepared at the following levels: 1,5; 3; 6; 9; 12; 15; and 18 µM. One ml of each solution was reacted with 2 ml of TCA 20% and 2 ml of TBA 1% in a glacial acetic acid 50%. The mixture was heated at 100°C for 10 minutes, centrifuged at 3,000 rpm for 15 mininutes. The absorbance of each level of TMP was spectrophotometrically measured at 532 nm. Distilled water was applied as blank solution.

Determination of α-glucosidase inhibitory activity (Sancheti et al. 2009). Inhibition against α-glucosidase (Sigma-Aldrich, Singapore) was tested, using p-nitrophenyl-α-D glucopyranoside (p-NPG) as substrate. In this experiment, substrate p-NPG was hydrolyzed by α-glucosidase to form glucose and pnitrophenol that produces yellow color. Microplate reader was used to check color changes spectrophotometrically at 410 nm. The measurement results were then used to calculate $IC_{50,}$ representing concentration of the extract able to inhibit 50% of enzyme activity.

Enzyme solution was made by dissolving 1 mg of α-glucosidase in 100 ml of buffer phosphate (pH 7) containing 200 mg of bovine serum albumin (BSA). Prior to use, one ml of enzyme solution was diluted 25 times using buffer phosphate (pH 7) 100 mM containing 200 mg of BSA. The reagent mixture was presented in Table 1, consisting of 25 of µL p-NPG 20 mM as substrate, 50 µL of buffer phosphate (pH 7) 100 mM, and 10 µL of sample in DMSO 1% (b/v) (concentration: 10, 100, 250, 500, 1,000, 2,500, 5,000, and 10,000 µg/ml) and 25 µL of enzyme. The reagent was incubated at 37°C for 30 minutes, and the enzymatic reaction was stopped by adding 100 µL of Na_2CO_3 200 mM. Spectrophotometric measurement was used to check absorbance at wavelength of 410 nm using microplate reader.

Negative control represented the reaction between substrate and enzyme in the absence of inhibitor, while blank solution represented reaction system in absence of both enzyme and inhibitor. All experimental units were made at triplicates. The % inhibition was calculated as follows:

$$\%Inhibition = \frac{control\ absorbance - sample\ absorbance}{control\ absorbance} \times 100\%$$

Acarbose was applied as positive control, acting as inhibitor of α-glucosidase. Acarbose was dissolved in buffer solution and HCL 2 N (1:1), at various concentrations: 0.10; 0.50; 1.00; 5.00; and 10.00 ppm (each 10 µL). The solution was reacted with reagent mixture, with similar procedure as tested samples. The absorbance was read using microplate reader at 410 nm.

Table 1. Reaction system of α-glucosidase inhibition

Solution	Blank (µL)	Control (µL)	S_o (µL)	S_1 (µL)
Extract	-	-	10	10
DMSO	10	10	-	-
Buffer	75	50	75	50
Substrate	25	25	25	25
Enzyme	-	25	-	25
Incubation at 37°C 30 minutes				
Na_2CO_3	100	100	100	100

Note: S_o = control; S_1= tested sample

Data analysis

Data were expressed as mean±standard deviation, and evaluated statistically using One-Way Analysis of Variance (ANOVA) in IBM SPSS Statistics 22. The significant difference among means was compared using Duncan test at α=0.05. IC_{50} was calculated using linear regression, plotting % inhibition vs ln concentration.

RESULTS AND DISCUSSION

A previous report showed that moisture content of mulberry leaf reached 7.26±0.76%, with a yield of 24.52%. The desirable results of experiment could be achieved due to low moisture level of the simplicia, i.e. <10% (BPOM 2014).

In this research, ethanol 65% was used as solvent since some studies suggested that ethanolic extracts of the mulberry plant is the best for significantly reducing glucose level. Ethanol 65% exhibited a higher efficiency in extraction of active compounds in mulberry leaf, approximately twice higher than aqueous solvent (Jeszka-Skowron *et al*. 2009). Further findings are also reported that ethanol and methanol at concentration of 40%–80% can isolate more polyphenol, flavonol, glycoside, and flavonoid over other solvents such as water, absolute ethanol and methanol.

Phytochemical Profile of Mulberry Leaf Extract

As presented in Table 2, the extract was confirmend to contain flavonoid, tanin and steroid, evidenced by positive result, while other compounds such as alkaloid, saponin, and alkaloid were confirmed negative.

Table 2. Results of phytochemical analysis on mulberry leaf extract

Compounds tested	Results
Alkaloid	-
Flavonoid	+
Tannin	+
Saponin	-
Steroid	+
Triterpenoid	-

Positive sign (+) indicates that the compound is present in the extract; Negative sign (-) indicates absence of the compound in the studied extract

Agustina *et al*. (2014) found that ethanolic extract of mulberry leaf was confirmed to contain quercetin and anthocyanin. In addition, the 45-days aged leaf was also known to have β-carotene at concentration of 2,04 μg/g. Another report showed that mulberry fruit possessed antioxidant properties reaching up to 86.79% based on DPPH assay (Natic *et al*. 2015). In other parts of the plant, ethanolic extract of mulberry's twigs and root barks was also confirmed to exert antioxidant and anti-tyrosine activity, in which the twig extract showed a stronger activity (Chang *et al*. 2011).

Incubation Time

Determination of optimum incubation time was based on conjugated diene method. As depicted in Figure 1, absorbance tends to cotently increase. This is due to the generation of carbon radicals during propagation phase as well as continuous reactions (Allouche *et al*. 2010). The increment of absorbance from day 0 to day 4 could indicate formation of conjugated diene hydroperoxide reaching maximum level. After this period, the absorbance showed a fluctuative condition, suggesting that conjugated diene hydroperoxides began to decompose, which in turn forms malonaldehyde (MDA) as product of lipid peroxidation.

We also found that the absorbance was recorded to rise in day 6, which could be ascribed to temperature level and less stable oxygen during period of incubation. Compared to absorbance in day 4, the value in day 6 was lower; thus, incubation time was considered to reach maximum level for 4 days. Based on this finding, analysis of potential antioxidant could be carried out about 2 days after optimum production of conjugated diene hydroperoxide. This is understandable that, in day 6, most hydroperoxide compounds were decomposed to form MDA.

Formation of conjugated dienes is influenced by several factors, such as heat, light, pH, oxygen, metal ions, and lipid radicals.

Oxidation of Linoleic Acid

Figure 2 showed that the highest concentration of MDA was attributed to negative control. This is clear that MDA radicals are extensively produced due to absence of antioxidant compounds. In this case, high quantity of MDA is a result of oxidation process towards linoleic acid.

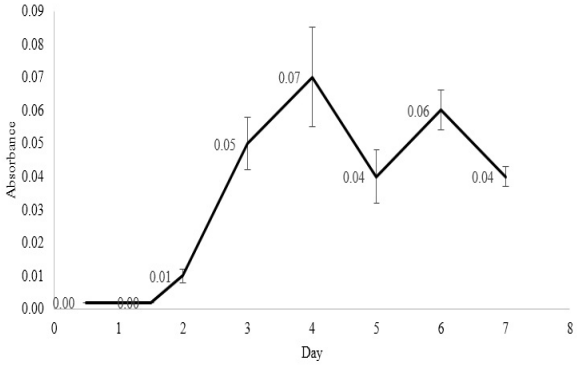

Figure 1. Changes in linoleic acid absorbance during 7 days of incubation time

The opposite result was found in group of positive control (α-tocopherol), in which the formation of MDA was significantly lower compared to other groups (α=0.05), reaching up to 3.08 with inhibitory activity of 62.06% (exhibited in Figure 3). This discrepancy may result from some factors, such as purity and quality of α-tocopherol, as well as incubation time. However, the MDA concentration was remarkably altered by mulberry leaf ethanolic extracts. Administration of the extract at dose of 125, 200, 500, and 1,000 ppm caused a meaningful effect on level of MDA, resulting in inhibition of 41.21%; 45.33%; 44.20%; and 36.00%, respectively. Meanwhile, Alfarabi *et al*. (2010) investigated the linoleic acid inhibition generated by extract of Piper crocatum leaves at 25, 50, 75, 100, 200 ppm dose, yielding inhibition of 44.31%, 56.30%, 63.00%, 72.68% and 80.4% respectively. In this present study the % of inhibition up to 200 ppm dose of showed the highest alteration. Based on this finding, the recommended concentration of the extract was 200 ppm. However, we need to emphasize that statistical evaluation demonstrated a non sisgnificant difference in the level of MDA as well as in % inhibition among mulberry extract treatments. Additionally, our data confirmed that α-tocopherol, as positive control, was noted as the strongest inhibitor over all other groups of treatments in this experiment.

Research focusing on antioxidant properties of mulberry leaf has been discussed by other researchers. Katsube *et al.* (2009) reported the antioxidant activity of mulberry leaf ethanolic extract against oxidation of LDL. The leaf extract was isolated by ethanol 60% as solvent, in which quercetin and rutin were found as the predominant flavonol glycosides. Jezka-Skowron *et al*. (2014) revealed that mulberry leaf extracted by ethanol 65% contained an appreciable amount of total phenolic, being 20% higher than sample extracted by acetone, while also producing lower TBA value. All of these reports could enrich the scientific evidence of mulberry leaf as a source of antioxidative compounds.

Inhibitory activity against α-glucosidase

Our experiment successfully confirmed the inhibitory activity of mulberry leaf extracts

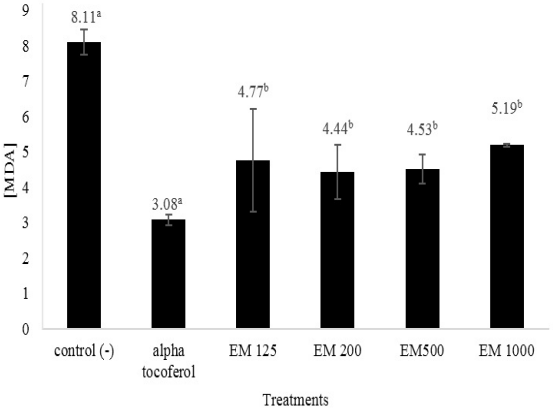

Figure 2. Effects of various treatments on MDA concentration. EM: extract ethanol mullberry.Those with different letters differ significantly at p<0.05

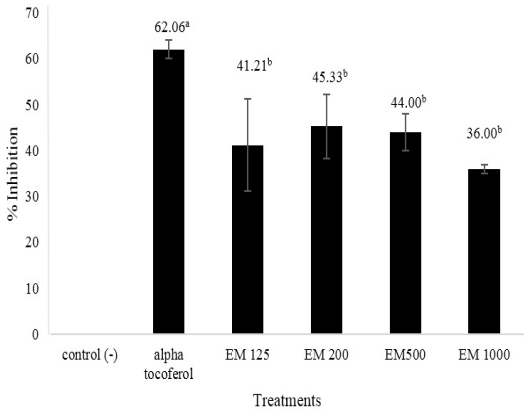

Figure 3. Effects of various treatments on % inhibition. EM: extract ethanol mullberry. Those with different letters differ significantly at p<0.05

towards α-glucosidase, as exhibited in Figure 4. In this case, inhibitory activity of the extract was expressed as IC_{50}, reaching up to 309.82±5.72 µg/ml (Table 3). The value of IC_{50} from mulberry leaf extracts was higher than that from acarbose (0.25±0.02 µg/ml), meaning that acarbose was much stronger as inhibitor over the studied extracts.

Inhibitory properties of mulberry leaf extracts against α-glucosidase are linked to presence of bioactive compounds such as alkaloid which is present in form of 1-deoxynojirimycin (DNJ). As reported by Kwon *et al*. (2011), DNJ acts as competitive inhibitor against α-glucosidase, thereby causing the reduction of glucose level after carbohydrate intake (postprandial hyperglycemia). Another study also found that chemical structure of DNJ is identic to D-glucose; therefore, it could alleviate absorption of D-glucose in the walls of small intestine (Voss *et al*. 2007).

Regarding to phytochemicals, it is noteworthy that alkaloid is not found according to qualitative experiment, which caused the low content of DNJ. Absence of alkaloid in the studied extract could be the major reason of the weak inhibition against α-glucosidase. Besides alkaloid, flavonoid is also reported as a significant compound responsible for inhibition of α-glucosidase (Kazeem *et al*. 2013). Flavonoind compound may exist in many forms, such as rutin, quercetin, and chlorogenic acid, in which they are scientifically confirmed able to inhibit α-glucosidase (Hunyadi *et al*. 2012). Previously, in vitro experiments reported that flavonoid and polyphenol are bioactive compounds that enable to reduce activity of α-glucosidase in intestine and α-amylase pancreatic (Koh *et al*. 2010, Pereira *et al*. 2011).

Furthermore, inhibition of such enzymatic actions could be performed by other sources. Yilmazer-Musa *et al*. (2012) investigated the inhibition of α-glucosidase generated by extracts of grape seed, green tea, teavigo, and white tea, yielding IC_{50} value of 1.20; 0.50; 0.30; and 2.50 µg/ml, respectively. IC_{50} of rich grape pomace extract was 1,630 µg/ml, reported by Hogan *et al*. (2010); meanwhile, Rubilar *et al*. (2011) reported IC_{50} of various extracts, such as murta (Ugni molinae Turcz.) leaf and fruit (215.70 and 61.30 µg/ml, respectively), and maqui (Aristotelia chilensis) leaf and stem (2.40 and 189.40 µg/ml respectively). Gomathi *et al*. (2012) investigated IC_{50} of ethanolic extract of Evolvulus alsinoides, reaching value of 86 µg/ml. In this present work, the IC_{50} of mulberry leaf ethanolic extract was higher than that of other extracts reported. As conclusive remark, we need higher concentration of the extract enabling to generate a stronger inhibition against α-glucosidase.

Table 3. Comparison of IC_{50} value between ethanolic extract and acarbose

Inhibitors	IC_{50} (µg/ml)*
Mulberry leaf extract (by ethanol 65%)	309.82±5.72
Acarbose	0.25±0.02

The mark * means that data are expressed as mean±standard deviation (n=3)

CONCLUSION

Mulberry (*Morus alba* L.) leaf extract isolated by ethanol 65% could alleviate production of MDA. Some bioactive compounds (i.e. flavonoid, tannin,steroid) present in the extract are responsible for bioactivity of the extract. Moreover, the extract was also confirmed capable of producing inhibitory action against α-glucosidase, although the inhibition was lower compared to acarbose.

ACKNOWLEDGEMENT

The authors would like to thank Department of Biochemistry, Faculty of Mathematics and Natural Sciences and Laboratory of Department of Food Science and Technology, Faculty of Agricultural Engineering and Technology, IPB University for research facilities. The authors have no conflict of interest.

REFERENCES

[AOAC] Association of Official Analytical Chemist. 2012. Official Method of Analysis of the Association of Official Analytical of Chemist. Arlington: The Association of Offiicial Anaytical Chemists.

Alfarabi M, Bintang M, Suryani, Safithri M. 2010. The Comparative Ability of Antioxidant Activity of Piper crocatum in Inhibiting Fatty Acid Oxidation and Free Radical

Scavenging. Hayati J Biosci. 17(4):201–204. https://doi.org/10.4308/hjb.17.4.201.

Agustina L, Mustabi J, Jamilah. 2014. Zat Bioaktif dan Daya Hambat Antibakteri Daun Murbei. [Proceeding] Seminar Nasional Agribisnis (page 1–6), 9 September. Semarang: Univesitas Diponegoro.

Allouche Y, Beltrán G, Gaforio JJ, Uceda M, Mesa MD. 2010. Antioxidant and antiatherogenic activities of pentacyclic triterpenic diols and acids. Food Chem Toxicol 48(10):2885–2890. https://doi.org/10.1016/j.fct.2010.07.022.

Amrun M, Umiyah, Umayah E. 2007. Uji Aktivitas antioksidan ekstrak air dan ekstrak Metanol Beberapa Varian Buah Kenitu (*Chrysophyllum cainito* L.) dari daerah Jember. Berk Penel Hayati 13:45–50.

Arabshahi-Delouee S, Urooj A. 2007. Antioxidant properties various solvent extract of mulberry (*Morus indica* L.) leaves. Food Chem 102(4):1233-1240. https://doi.org/10.1016/j.foodchem.2006.07.013.

[BPOM] Badan Pengawas Obat dan Makanan. 2014. Peraturan Kepala Badan Pengawas Obat dan Makanan Republik Indonesia Nomor 12 Tahun 2014 Tentang Persyaratan Mutu Obat Tradisional. Jakarta: BPOM.

Barclay AW, Petocz P, McMillan-Price J, Flood VM, Prvan T, Mitchell P, Brand-Miller JC. 2008. Glycemic index, glycemic load, and chronic disease risk–ameta-analysis of observational studies. Am J Clin Nutr 87(3):627–637. https://doi.org/10.1093/ajcn/87.3.627.

Castellano JM, Guinda A, Delgado T, Rada M, Cayuela JA. 2013. Biochemical basis of the antidiabetic activity of oleanolic acid and related pentacyclic triterpenes. Diabetes 62(6):1791–1799. https://doi.org/10.2337/db12-1215.

Chang LW, Juang LJ, Wang BS, Wang MY, Tai HM, Hung WJ, Chen YJ, Huang MH. 2011. Antioxidant and antityrosinase activity of mulberry (*Morus alba* L.) twigs and root bark. Food Chem Toxicol 49(4):785–790. https://doi.org/10.1016/j.fct.2010.11.045.

Chung HI, Kim J, Kim JY, Kwon O.2013. Acute intake of mulberry leaf aqueous extract affects postprandial glucose response after maltose loading: Randomized double-blind placebo-controlled pilot study. J Funct Foods 5(3):1502–1506. https://doi.org/10.1016/j.jff.2013.04.015.

Efendi R, Damaynthi E, Kustiyah L, Kusumorini N.2010. Pengendalian kadar glukosa darah oleh the hijau dan atau the daun murbei pada tikus diabetes. Jurnal Gizi dan Pangan 5(2): 87–94. https://doi.org/10.25182/jgp.2010.5.2.87-94.

Esterbauer H, Striegl G, Puhl H, Rothene er M. 1989. Continuous monitoring of in vztro oxidation of human low density lipoprotein. Free Radic Res Commun 6(1):67–75. https://doi.org/10.3109/10715768909073429.

Gomathi D, Kalaiselvi M, Ravikumar G, Sophia D, Gopalakrishnan VK, Uma C. 2012. Secondary metabolite credentials of *Evolvulus alsinoides* by high performance thin layer chromatography (HPTLC). J Biomed Res 26(4):295–302. doi: 10.7555/JBR.26.20110128.

Huang HP, Ou TT, Wang CJ. 2013. Mulberry and its bioactive compounds, the chemoprevention effects and molecular mechanisms in vitro and in vivo. J Tradit Complement Med. 3(1):7–15. https://doi.org/10.4103/2225-4110.106535.

Harborne JP. 1987. Metode Fitokimia. (Padmawinta K, Soediro I, penerjemah). Bandung: ITB.

Hunyadi A, Martins A, Hsieh TJ, Seres A, Zupko I. 2012. Chlorogenic acid and rutin play a major role in the in vivo antidiabetic activity of *Morus alba* leaf extract on type II diabetic rats. Plos One 7(11): e50619 1–7. https://doi.org/10.1371/journal.pone.0050619.

Hogan S, Zhang L, Li J, Sun S, Canning C, Zhou K. 2010. Antioxidant rich grape pomace extract suppresses postprandial hyperglycemia in diabetic mice by specifically inhibiting alpha-glucosidase. Nutr Metabo 7(71):1–9. https://doi.org/10.1186/1743-7075-7-71.

Jeszka-Skowron M, Kobus J, Flaczyk E. 2009. Evaluation of antioxidant activity of Morus alba leaf extracts. Bromatol Chem Toksykol. 42:2791–2796.

Jezka-Skowron M, Flaczyk E, Jeszka J, Krejpcio Z, Krol E, Buchowski MS. 2014. Mulberry leaf extract intake reduces hyperglycaemia

in Streptozotocin (STZ)-induced diabetic rats fed high-fat diet. J Funct Foods 8:9–17. https://doi.org/10.1016/j.jff.2014.02.018.

Katsube T, Tsurunaga Y, Sugiyama M, Furuno T, Yamasaki Y. 2009. Effect of air-drying temperature on antioxidant capacity and stability of polyphenolic compounds in mulberry (*Morus alba* L.) leaves. Food Chem 113(4):964–969. https://doi.org/10.1016/j.foodchem.2008.08.041.

Kazeem MI, Raimi OG, Balogun RM, Ogundajo AL. 2013. Comparative study on the α-amylase and α-glucosidase inhibitory potential of different extracts of *Blighia sapida* Koenig. Am J Res Commu 1(7): 178–192.

Kikuzaki H dan Nakatami N. 1993. Antioxidant effects of some ginger constituens. J Food Sci 58(6):1407–1410. https://doi.org/10.1111/j.1365-2621.1993.tb06194.x.

Koh LW, Wong LL, Loo YY, Kasapis S, Huang D. 2010. Evaluation of different teas against starch digestibility by mammalian glycosidases. J Agric Food Chem 58(1):148–154. https://doi.org/10.1021/jf903011g.

Kwon HJ, Chung JY, Kim JY, Kwon O. 2011. Comparison of 1-deoxynojirimycin and aqueos mulberry leaf extract with emphasis on postprandial hypoglycemic effects: in vivo and in vitro studies. J Agric Food Chem 59(7): 3014–3019. https://doi.org/10.1021/jf103463f.

Natic MM, Dabic DC, Papetti A, Aksic MMF, Ognjanov V, Ljubojevic M, Tesic ZL. 2015. Analysis and characterisation of phytochemicals in mulberry (*Morus alba* L.) fruits grown in Vojvodina, North Serbia. Food Chem 171:128–136. https://doi.org/10.1016/j.foodchem.2014.08.101.

Pereira DF, Cazarolli LH, Lavado C, Mengatto V, Figueiredo MSRB, Guedes A, Pizzolatti MG, Silva FRMB. 2011. Effects of flavonoids onα-glucosidase activity: Potential targets for glucose homeostasis. Nutrition 27(11–12):1161–1167. https://doi.org/10.1016/j.nut.2011.01.008.

Rubilar M, Jara C, Poo Y, Acevedo F, Guitierrez C, Sineiro J, Shene C. 2011. Extracts of maqui (*Aristotelia chilensis*) and murta (*Ugni molinae* Turcz.): Source of antioxidant compounds and α-glucosidase/α-amylase inhibitors. Am J Pharmacol Toxicol 59(5):1630-1637. http://pubs.acs.org/doi/abs/10.1021/jf103461k.

Sancheti S, Sancheti S, Yum SS. 2009. *Chaenomeles sinensis*: A potent α-and β-glucosidase inhibitor. American J Pharm Toxicol 4(1): 8–11.

Voss AA, Diexz-Sampedro A, Hirayama BA, Loo DDF, Wright EM. 2007. Imino sugars are potent agonists of the human glucose sensor $SGLT_3$. Mol Pharmacol 71(2):628–634. https://doi.org/10.1124/mol.106.030288.

Yilmazer-Musa M, Griffith AM, Michels AJ, Schneider E, Frei B. 2012. Grape seed and tea extracts and catechin 3-gallates are potent inhibitors of α-amylase and α-glucosidase activity. J Agric Food Chem 60(36):8924-8929. https://doi.org/10.1021/jf301147n.

[WHO] World Health Organization. 2015. Media Centre. Diabetes. Fact sheet No 312. World Health Organization. https://www.who.int/nmh/publications/fact_sheet_diabetes_en.pdf. [Accessed 20 June 2015].

Available online: http://journal.ipb.ac.id/index.php/jgizipangan ***J. Gizi Pangan, March 2020, 15(1):53-62***
Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019 *DOI: 10.25182/jgp.2020.15.1.53-62*
ISSN 1978-1059 EISSN 2407-0920

Predictors of Prenatal Breastfeeding Self-Efficacy in Malaysian Women: A Cross-Sectional Study

Syahrul Bariah Abdul Hamid[1*], Nurshazlyana Mohamad Zaidi[1]
Mother, Infant & Young Child Nutrition (MiChild) Research Group
Centre of Nutrition of Dietetics, Faculty of Health Science, Universiti Teknologi MARA (UiTM)
Cawangan Selangor, Kampus Puncak Alam, Selangor, 42300 Puncak Alam, Selangor, Malaysia

ABSTRACT

Mothers with better self-efficacy are tend to have intent to breastfeed, initiate early and have lengthier of exclusive breastfeeding. This study aimed to evaluate the level of breastfeeding self-efficacy and to investigate the determinants of breastfeeding self-efficacy among pregnant mothers. A total of 180 expecting mothers were recruited in this cross sectional study from chosen Maternal and Child Health Clinics in Selangor. Self-administered questionnaires of Iowa Infant Feeding Attitude Score (IIFAS) and Breastfeeding Self-Efficacy (BSES-SF) were used to attain information on maternal attitudes and knowledge of breastfeeding and breastfeeding self-efficacy. Findings showed subjects had high level of breastfeeding self-efficacy (mean 51.79±11.94) and majority of them had fair knowledge in breastfeeding. Breastfeeding self-efficacy is found associated with number of children, while, residential area, occupation and household income were associated with breastfeeding knowledge ($p<0.05$). The best-fit regression analysis revealed three variables that explained 41.0% of the variance in breast feeding self-efficacy among expectant mothers. They were being housewife, multiparous and had positive breastfeeding attitudes ($p<0.05$). For that reason, healthcare providers can tactically identify women vulnerable to low breastfeeding self-efficacy by providing early intervention through increasing the awareness and knowledge in breastfeeding during prenatal and antenatal.

Keywords: breastfeeding knowledge, breastfeeding self-efficacy, theory planned behaviour

INTRODUCTION

Breastfeeding can improve development, well-being and survival of children. Breast milk not only defenses the well-being during childhood, but also offers long term protection throughout life (Binns *et al*. 2016). There is a vast evidence on both long and short term benefits of breastfeeding for mothers and children and its positive effects on supporting mental, physiological health and development through nutrition in breast milk in reducing morbidity and mortality rates in children especially during the first few months of life (Victora *et al*. 2016; Eidelman *et al*. 2012).

In Malaysia, despite numerous supports and campaigns piloted by the government, the breastfeeding rates are still lower than the rate recommended by WHO (2019). Despite the fact that the breastfeeding rates have increased since year 2006, according to the National Health and Morbidity Survey, only 47.1 % and 98.1% of infants under 6 months were exclusively breastfed and ever breastfed respectively (Institute for Public Health 2016). In addition, prevalence of timely initiation was 65.3% and the continued prevalence of breastfeeding up to two years was only 39.4%. However, the rates were far below than the recommended by WHO regardless of numbers of awareness programs on benefits of breastfeeding extensively moderated by the government.

Present study also has observed the relationship between many variables and their impact on breastfeeding outcomes; timely initiation, duration of breastfeeding and exclusivity of breastfeeding (Castro *et al*. 2017). Key factors such as maternal attitudes, breastfeeding self-confidence and breastfeeding self-efficacy demonstrate a positive relationship with sustained

[*]**Corresponding Author:** tel: +60332584382, email: syahrulbariah@warga.uitm.edu.my

breastfeeding and some evidence exists that these variables may be modifiable towards experiences in breastfeeding (Leahy-Warren *et al*. 2014; Meedya *et al*. 2010).

Breastfeeding self-efficacy denotes to a mother's ability or confidence to breastfeed her newborn as she perceived and this inflict her choices on early initiation of breastfeeding, exclusivity and duration of breastfeeding as well as how she will tackle any breastfeeding issues (Dennis 1999). To increase breastfeeding self-efficacy, a mother must have the beliefs that breastfeeding will produce positive outcomes and also mothers must display the confidence in executing the specific behaviour which is breastfeeding. Past exposure to breastfeeding experiences plays an important roles to explain attitude, subjective norms and self-efficacy of mothers to their next breastfeeding practice as intention to breastfeed were predicted by subjective norms and attitude mothers to the formula-feeding and also mothers breastfeeding experience (Bartle & Harvey 2017).

Higher perceived breasfeeding self-efficacy mothers tend to initiate breastfeeding early and continue to breastfeed despite facing challenges during breastfeeding, meanwhile, mothers with a lower perceived self-efficacy may do not have the intention, delay the initiation or wean in advance due to lack of confidence or ineffective coping skills. Therefore, mothers with have higher self-efficacy or breastfeeding control are tend to have positive breastfeeding outcomes (Zhu *et al*. 2016).

According to Dennis (1999), who developed Theory of Breastfeeding Self-Efficacy, the choice to breastfeed is influenced by four processes which are; if the mother decides to breastfeed or not, how much effort that the mother needs, is the mother has a self-encouraging pattern of thinking and how the mother tackles the problem of breastfeeding difficulties (Dennis 1999). Bandura (1977) suggested that social supports, personal experience, vicarious involvement, verbal encouragement and emotional and physiological condition may influence a mother's efficacy to breastfeed. Higher perceived social support mothers were associated with higher level of breastfeeding self-efficacy and linked to increased intention to breastfeed, early initiation of breastfeeding and longer duration of breastfeeding (Mannion *et al*. 2013; Yang *et al*. 2016; Mirghafourvand *et al*. 2018).

Therefore, breastfeeding self-efficacy during pregnancy could be a predictor of positive breastfeeding outcomes. It also can be an indicator to identify those who need further assistance to warrant continuance of breastfeeding. However, no study has been carried out to measure breastfeeding self-efficacy level and investigate the predictors of breastfeeding self-efficacy among Malaysian expectant mothers.

METHODS

Design, location & time

This cross sectional study was conducted among expecting mothers who attending chosen Maternal and Child Health Clinics in Selangor, Malaysia.

Convenience sampling was used in this study with inclusion criteria as follows; eligible pregnant mothers and willing to participate in the study, singleton pregnancy, age between 18–41 years old, could speak and read in English or Malay. They will be excluded from participation if they had serious medical or obstetric conditions and multiple pregnancies (twins, triplets).

This study was conducted in Selangor from September to November 2017. Selangor is one of the most populous state located in Peninsular Malaysia. Two Maternal and Child Health clinics in Kuala Selangor district were chosen for this study.

Sampling

The sample size was calculated based on any given population using Krejcie and Morgan (1970) table in determining sample size. Given the nearest population size of 360, the determined sample size was 180 subjects. Thus, for the purpose of this study, 180 pregnant mothers were recruited during their antenatal examinations.

Data collection

The data collection was in the form of self-administered questionnaire. Data included were socio-demographic, maternal attitudes towards infant feeding and breastfeeding self-efficacy.

This study used a validated Breastfeeding Self Efficacy Scale-Short Form (BSES-SF) that was translated into Malay language instrument to evaluate level of breastfeeding self-efficacy among expectant mothers. The Cronbach's Alpha value for Malay version of antenatal BSES-SF questionnaire was 0.94 (Husin *et al*. 2017). This Breastfeeding Self Efficacy Scale-Short Form (BSES-SF) was originally has 33 questions and has been simplified into 14 questions (Dennis 2003). In this instrument, all questions are begin with phrase "I can always". The score uses the Likert scale which start from 1 for not confident at all to 5 for very confident. Higher marks directed higher levels of breastfeeding self-efficacy. Furthermore, outcome expectancy and self-efficacy expectancy are two components in self-efficacy concept. In order to predict how long mothers will continue to breastfeed, Breastfeeding self-efficacy Scale (BSES) is the best predictor that can be used. In BSES-SF, the minimum and maximum scores were 14 and 70 respectively. The scores of less than 50 indicates higher risk for breastfeeding cessation. The information used in BSES-SF is about the confidence in producing adequate breast milk, exclusive breastfeeding, using formula milk, correct latching, breastfeeding in public and satiety of the baby.

The Iowa Infants Feeding Attitude Scale (IIFAS) consists of 17 questions to measure the level of attitude in breastfeeding. The questions were divided into positive to breastfeeding and positive to artificial feeding. Scoring for questionnaires is based on a Likert scale from 1 to 5 (1=absolutely disagree, 2=disagree, 3=no idea, 4=agree, and 5=absolutely agree). The IIFAS has been translated and validated in Malay language and have been used among mothers in Malaysia (Shukri *et al*. 2017). The Cronbach's alpha for this instrument was 0.7–0.8 (Karande & Parkar 2012). Greater scores directed to a more positive attitude to breastfeeding. Questions of 1, 2, 4, 6, 8, 10, and 13 were scored reversely. This questionnaire is reliable and valid, the Cronbach's alpha was stated between 0.85-0.86 (Mora *et al*. 1999). The total attitude scores range from 17 (indicating positive attitudes towards artificial feeding) to 85 (reflecting positive attitudes towards breastfeeding) while the score of 51 indicated a neutral attitude.

Permissions were attained from the subjects by written informed consent. The subjects were informed that the participation was voluntarily and their administered data would be assured confidentially for the purpose of the study only. This study was approved by UiTM Research Ethics Committee (REC/151/17) and Medical Research Ethics Committee, Ministry of Health (NMRR-17-1299-36056).

Data analysis

Data is analyzed using the Statistical Package for Social Sciences (SPSS) version 21.0 software. Descriptive statistics were calculated for socio-demographic data such as age, ethnicity and household income. Normality test was carried out using Kolmogorov-Smirnov Test of Normality. Differences of breastfeeding self-efficacy among mothers from different socio-demographic characteristics were analysed by one-sided independent sample t-test or one way ANOVA. Significant variables ($p<0.05$) were included in the multiple linear regressions to investigate the predictors of the breastfeeding self-efficacy.

RESULTS AND DISCUSSION

Sample characteristics

Table 1 showed a total 180 pregnant women took part in this study. The age of participants diversed from 19 and 41 years old. More than half of the expectant mothers (69.4%) were aged between 19 to 30 years old. Most participants were Malay (91.7%) and only (8.3%) are non-Malay. More than half (62.8%) of our participants were working mothers. A total of 65.6% of respondents received education up to (more than 12 years of education) and 58.3% of the mothers had children more than one. The majority (81.1%) of the mothers were neutral and only 16.7% of the mothers had positive attitude towards breastfeeding.

In addition, this study reported that majority mothers who had high knowledge, imposed more positive attitude towards breastfeeding with mean score of 63.32±5.96. The finding is supported by a randomized controlled study done to evaluate the

effectiveness of prenatal breastfeeding workshop. It is suggested mothers with higher knowledge on breastfeeding practices and benefits of breast milk had higher possibilities to have higher score of breastfeeding self-efficacy and resulted to longer duration of exclusive breastfeeding (Noel-Weiss *et al*. 2006). Similar finding from Bartle and Harvey also found higher maternal breastfeeding self-efficacy during pregnancy is associated with higher intention to breastfeed and therefore, predicted better breastfeeding outcomes (Bartle & Harvey 2017).

There was a significant statistical association between breastfeeding self-efficacy and parity in this study as seen in Table 1. This finding aligned with study done by Draman and friends in 2017, they found that previous breastfeeding practice had a significant association with current exclusive breastfeeding practices. However, results of a study by Bartle and Harvey (2017) on mothers who were multiparous showed that past experience of birth and breastfeeding practices predicted attitudes and subjective norms that were in favor of breastfeeding but not to their breastfeeding self-efficacy. While in the other hand, having experienced difficulties in breastfeeding imposed lower breastfeeding self-efficacy.

Breastfeeding self-efficacy

The objective of this present study was to assess the level of breastfeeding self-efficacy and investigate its determinants among pregnant mothers. Table 2 showed mean, median and standard deviation values for each question on the BSES-SF. The present study found that the mean score of breastfeeding self-efficacy among expectant mothers was 51.79 (SD=11.94). A similar pattern of mean scores were observed in two psychometric analysis studies which were 56.20 (SD=8.75) and 58.52, respectively (Husin *et al*. 2017; Alus-Tokat *et al*. 2010). These mothers had high self-efficacy towards breastfeeding and it is suggested could be due to more than 50% of the expecting mothers had more than one child and they may have past exposure in breastfeeding as discussed in several other studies (Mirghafourvand *et al*. 2018; Husin *et al*. 2017).

The three items that had the uppermost scores were 'confidence to be able to breastfeed exclusively', 'coping with breastfeeding' and 'personal desire to breastfeed'. The items that had the bottommost score were 'continue to breastfeed at every feed' and 'finishing feeding my baby before switching to the other breast', suggesting mothers had lowest confidence in these perspectives.

Further investigation on the breastfeeding self-efficacy items in the present study proposed that mothers had minimal confidence on appropriate timing to finish feeding and either their milk supply is adequate to sustain with the baby's demand. The findings were similar to Finnish and Chinese studies which highlighted the mothers' concern on milk insufficiency to achieve the baby's demand and breastfeeding without supplementing with formula milk (Yang *et al*. 2016; Koskinen *et al*. 2014).

Predictors of breastfeeding self-efficacy

Table 3 shows the variables that have significant relationship with BSES-SF scores which were counted in the stepwise regression model. The best-fit regression model discovered that there are three variables that explained 41.0% of the variance in BSES-SF scores. The final determinants for breastfeeding self-efficacy among expectant mothers were having more than one child, being housewife and positive attitude towards breastfeeding. Therefore, the predicted of expected breastfeeding self-efficacy is written in this equation: Y (BSES)=21.00 + (0.579 x positive towards breastfeeding) + (4.826 x housewife) + (4.208 x multiparous). From this equation, each one score increase in breastfeeding self-efficacy is associated with a 0.579 increase with positive attitude towards breastfeeding, a 4.826 increase with being housewife and 4.208 increase in mothers with multiparous. These variable statistically significant in predicting breastfeeding self-efficacy, F (8.171) = 4.323, $p<0.05$, R2=0.410.

In the present study, it is understood that parity was a predictor of breastfeeding self-efficacy. In Table 1 depicted multiparous mothers (54.05±10.82) had higher breastfeeding self-efficacy compared to uniparous mothers (48.60±12.76). This is also supported by a

Table 1. Characteristics of respondents (n=180)

Socio-demographic factors	n	Percentage (%)	Mean (SD)	p value
Age (years)				0.292
19–31	125	69.4	51.16 (12.18)	
31–41	55	30.6	53.20 (11.35)	
Race				0.325
Malay	165	91.7	52.04 (11.67)	
Non-Malay	15	8.3	48.87 (14.73)	
Area of residency				0.613
Klang	148	82.2	51.99 (12.19)	
Kuala Selangor	32	17.8	50.81 (10.81)	
Employment status				0.064
Working	113	62.8	50.51 (13.36)	
Housewife	67	37.2	53.93 (8.75)	
Education level				0.570
<12 years	62	34.4	52.48 (10.75)	
>12 years	118	65.6	51.42 (12.55)	
Parity				0.020*
Uniparous	75	41.7	48.60 (12.76)	
Multiparous	105	58.3	54.05 (10.82)	
Level of income (Ringgit Malaysia, RM)				0.861
<RM3000	92	51.1	51.63 (10.74)	
>RM3000	88	48.9	51.94 (13.14)	
Attitude to infant feeding				0.000*
Positive to formula feeding	4	2.2	53.5 (9.33)	
Neutral	146	81.1	49.97 (11.87)	
Positive to breastfeeding	30	16.7	60.37 (8.57)	

*statistically significant p<0.05

study done by Husin *et al.*(2017), they agreed on new and uniparous mothers who had no breastfeeding experience tend to have limited confidence in breastfeeding compared to multiparous mothers with multiple children. This is also coincided with literature that breastfeeding self-efficacy linked to exposure to past personal breastfeeding experience compared to first-time mothers (Bartle & Harvey 2017). Personal experience of breastfeeding practices among mothers play an important role to explain attitude, subjective norms and self-efficacy of mothers to their next breastfeeding practices as intention to breastfeed were predicted by subjective norms and attitude of the mothers towards formula-feeding. It is also suggested that previous breastfeeding experience and prior exposure in breastfeeding predict one's intention and attitudes towards positive breastfeeding outcomes (Abdul Hamid & Yahya 2018).

Table 2. BSES-SF scores by mean, median and standard deviation (n=180)

BSES-SF	Mean	Median	Standard deviation
I can always determine that my baby is getting enough milk	3.82	4.00	1.11
I can always successfully cope with breastfeeding like I have with other challenging tasks	3.94	4.00	1.01
I can always breastfeed my baby without using formula as a supplement	3.56	3.00	1.15
I can always ensure that my baby is properly latched on for the whole feeding	3.99	4.00	0.99
I can always manage the breastfeeding situation to my satisfaction	3.87	4.00	1.07
I can always manage to breastfeed even if my baby is crying	3.58	4.00	1.10
I can always keep wanting to breastfeed	3.88	4.00	0.98
I can always comfortably breastfeed with my family members present	3.50	3.00	1.13
I can always be satisfied with my breastfeeding experience	3.69	4.00	1.16
I can always deal with the fact that breastfeeding can be time consuming	3.84	4.00	1.02
I can always finish feeding my baby on one breast before switching to the other breast	3.38	3.00	1.13
I can always continue to breastfeed my baby for every feeding	3.32	3.00	1.17
I can always manage to keep up with my baby's breastfeeding demands	3.69	4.00	1.08
I can always tell when my baby is finished breastfeeding	3.73	4.00	1.08

BSES-SF: Breastfeeding Self Efficacy Scale-Short Form

Our study found that mothers' breastfeeding self-efficacy was correlated to their perceived attitude towards breastfeeding. In the Table 1, pregnant mothers who had higher score in IIFAS and had positive attitude towards breastfeeding (60.37±8.57) had higher breastfeeding self-efficacy compared to those who were positive attitude towards formula-feeding (53.55±9.33) and were neutral on infant feeding (49.97±11.87). Higher score in IIFAS is associated with having good knowledge in breastfeeding (Dungy *et al*. 2008). Meanwhile, pregnant mothers who had more knowledge in breastfeeding displayed positive attitudes towards exclusive breastfeeding Utari *et al*. 2014) and is one of the predictor of breastfeeding intention (Abdul Hamid *et al*. 2017). Meanwhile, mothers with intention to exclusive breastfeed their newborns is linked to greater maternal confidence in breastfeeding and therefore, may foresee positive breastfeeding practices. Breastfeeding

Table 3. Predictors of breastfeeding self-efficacy among expectant mothers (n=180)

		Regression coefficients	p value
Intercept	21.00		
R^2 value, BSES*	0.410		
Parity		4.208	0.014*
Employment status		4.826	0.007*
Attitude		0.579	0.000*

Overal R^2 = 0.410; model fit: F=4.32
$p < 0.05$; statistically significant (Stepwise method)
Parity: 1=uniparous; 2=multiparous
Employment status: 1=employed; 2=housewife
Attitude: 1=ambivalent to infant feeding; 2=positive towards breastfeeding
*Breastfeeding self efficacy scale

intention is defined as a mother's anticipated duration of breastfeeding, and this may predict the actual breastfeeding duration. Mothers with moderate to low level of breastfeeding self-efficacy tend to wean breastfeeding earlier two times higher than mothers with high self-efficacy. Mothers with high self-efficacy had intents to breastfeed for a lengthier period and this association is correlated well with parity whereby the exclusivity period was longer for the second child (Kronborg *et al*. 2018).

Employment status was also a predictor of breastfeeding self-efficacy. In Malaysia, percentage of labor force participation by female is 50.98 % (The Global Economy 2018), meanwhile, the prevalence of breastfeeding among working mothers were high at 97.6% (Rashid *et al*. 2018). This study found that there is a correlation between breastfeeding support at workplace and breastfeeding self-efficacy. Working mothers tend to breastfeed their babies longer when their employer supporting breastfeeding practices by providing adequate facilities at work place such as breast pump and nursing room and also longer maternity leave (Alzaheb 2017).

To our knowledge, this present study is the first of its kind in Malaysia that discovered the predictors of breastfeeding self-efficacy among mothers during antenatal period. Breastfeeding self-efficacy is a modifiable factor, therefore early intervention should be carried out by targeting expectant mothers to improve breastfeeding rates (Brockway *et al*. 2017).

However, this present study has several limitations. This study was piloted in government health clinics. Majority of the participants came from average social class with high level of educational background. These findings may not be extrapolated to other settings with subjects who are less educated and from lower social class. On top of that, as of breastfeeding self-efficacy, it is self-reported and was taken only once from the mothers at various different trimesters. In addition, some predictors of breastfeeding self-efficacy was not taken into measurement such as past breastfeeding experience and social supports.

CONCLUSION

There are several autonomous variables that can influence maternal breastfeeding self-efficacy whether in form of modifiable factors or non-modifiable factors. This study suggested that determinants of breastfeeding self-efficacy among pregnant mothers in Malaysia are having positive attitude towards breastfeeding, being a housewife and had more than one child. Therefore, in order to increase breastfeeding rates, exposure to breastfeeding awareness and skill campaign should be given to expectant mothers especially mothers with their first child who have little experience in breastfeeding. In addition intervention to improve attitudes toward breastfeeding is also needed. Increasing supports from family and society as well as the employers by offering well-equipped breastfeeding facilities at workplace for working mothers are also crucial to increase the breastfeeding rate.

ACKNOWLEDGEMENT

This project was funded by Lestari Research Grant (600-IRMI/DANA5/3/ LESTARI(0101/ 2016) provided by Institute of Research Management and Innovation, MARA

University of Technology, Selangor, Malaysia. Special thanks also to the pregnant mothers who had spent their time and shared their information with the researcher throughout this study. The authors have no conflict of interest.

REFERENCES

Abdul Hamid SB, Yahya N. 2018. Knowledge, attitude, prior exposure and intention to breastfeed among undergraduate university students. Journal Of Clinical And Health Sciences 3(2):26–35. doi:10.24191/jchs.v3i2.7083.

Abdul Hamid SB, Chih HJ, Binns CW. 2017. Predictors of breastfeeding intention in Malaysia. Environment Behavioral Proceeding Journal 2(5): 161–167. https://doi.org/10.21834/ebpj.v2i5.693.

Alzaheb RA. 2017. Factors Influencing Exclusive Breastfeeding in Tabuk, Saudi Arabia. Clin Med Insights Pediatr 11:1–8. https://doi.org/10.1177/1179556517698136.

Bandura A. 1977. Self-efficacy: Toward a unifying theory of behavioral change. Psychological Review 84(2): 191–215. https://doi.org/10.1037/0033-295X.84.2.191.

Bartle NC, Harvey K. 2017. Explaining infant feeding: The role of previous personal and vicarious experience on attitudes, subjective norms, self-efficacy, and breastfeeding outcomes. Br J Health Psychol 22(4):763–785. doi:10.1111/bjhp.12254.

Binns C, Lee M, Low WY. 2016. The Long-term public health benefits of breastfeeding. Asia Pac J Public Health 28(1): 7–14. https://doi.org/10.1177/1010539515624964.

Brockway M, Benzies K, Hayden KA. 2017. Interventions to improve breastfeeding self-efficacy and resultant breastfeeding rates: A systematic review and meta-analysis. J Hum Lact 33(3):486–499. https://doi.org/10.1177/0890334417707957.

Castro RTA, Glover V, Ehlert U, Connor TG. 2017. Antenatal psychological and socioeconomic predictors of breastfeeding in a large community sample. Early Human Development 110:50–56. https://doi.org/10.1016/j.earlhumdev.2017.04.010.

Dennis CL. 1999. Theoretical underpinnings of breastfeeding confidence: A self-efficacy framework. J Hum Lact 15(3):195–201. https://doi.org/10.1177/089033449901500303.

Dennis CL. 2003. The breastfeeding self-efficacy scale: Psychometric assessment of the short form. J Obstet Gynecol Neonatal Nurs 32(6):734–744. https://doi.org/10.1177/0884217503258459.

Draman N, Mohammad N, Yusoff HM, Muhamad R. 2017. The decision of breastfeeding practices among parents attending primary health care facilities in suburban Malaysia. Journal of Taibah University Medical Sciences 12(5):412–417. https://doi.org/10.1016/j.jtumed.2017.05.005.

Dungy CI, McInnes RJ, Tappin DM, Wallis AB, Oprescu F. 2008. Infant feeding attitudes and knowledge among socioeconomically disadvantaged women in Glasgow. Matern and Child Health J 12:313–322. https://doi.org/10.1007/s10995-007-0253-9.

Eidelman AI, Schanler RJ, Johnston M. 2012. Breastfeeding and the use of human milk. Pediatrics 129(3):e827–e841. https://doi.org/10.1542/peds.2011-3552.

Husin H, Isa ZM, Latif KA, Ariffin R, Rahman SA, Ghazi HF. 2017. The Malay version of antenatal and postnatal breastfeeding self-efficacy scale-short form: Reliability and validity assessment. Malaysian Journal of Public Health Medicine. 17(2):62–69.

Institute for Public Health. 2016. National Health and Morbidity Survey 2016: Maternal and Child Health Volume II. Kuala Lumpur: Institute for Public Health, National Institutes of Health, Ministry of Health Malaysia.

Karande S, Parkar S. 2012. Do fathers' attitudes support breastfeeding? A cross-sectional questionnaire-based study in Mumbai, India. Indian JMed Res 66(1):30–39. doi:10.4103/0019-5359.110861.

Koskinen KS, Aho AL, HAnnula L, Kaunonen M. 2014. Maternity hospital practices

and breast feeding self-efficacy in Finnish primiparous and multiparous women during the immediate postpartum period. Midwifery 30:464–470. https://doi.org/10.1016/j.midw.2013.05.003.

Krejcie R V, Morgan DW. 1970. Determining sample size for research activities. Educ Psychol Meas 607–610. https://doi.org/10.1177/001316447003000308.

Kronborg H, Foverskov E, Vaeth M, Maimburg RD. 2018. The role of intention and self-efficacy on the association between breastfeeding of first and second child, a Danish cohort study. BMC Pregnancy Childbirth 18:454. https://doi.org/10.118.

Leahy-Warren P, Mulcahy H, Phelan A, Corcoran P. 2014. Factors influencing initiation and duration of breast feeding in Ireland. Midwifery 30(3): 345–52. https://doi.org/10.1016/j.midw.2013.01.008.

Mannion CA, Hobbs AJ, McDonald SW, Tough SC. 2013. Maternal perceptions of partner support during breastfeeding. Int Breastfeed J (8):4. http://dx.doi.org/10.1186/1746-4358-8-4.

Meedya S, Fahy K, Kable A. 2010. Factors that positively influence breastfeeding duration to 6 months: A literature review. Women and Birth 23(4): 135–145. https://doi.org/10.1016/j.wombi.2010.02.002.

Mirghafourvand M, Malakouti J, Mohammad-Alizadeh-Charandabi S, Faridvand F. 2018. Predictors of breastfeeding self-efficacy in Iranian women: A cross-sectional study. International Journal of Women's Health and Reproduction Sciences 6(3):380–385. 10.15296/ijwhr.2018.62.

Mora ADL, Russell DW, Dungy CI, Losch M, Dusdieker L. 1999. The Iowa infant feeding attitude scale: Analysis of reliability and validity. J Appl Soc Psychol 29(11):2362–2380. https://doi.org/10.1111/j.1559-1816.1999.tb00115.x.

Noel-Weiss J, Rupp A, Crag B, Bassett V, Woodend AK. 2006. Randomized controlled trial to determine effects of prenatal breastfeeding workshop on maternal breastfeeding self-efficacy and breastfeeding duration. J Obstet Gynecol Neonatal Nurs 35(5):616–624. https://doi.org/10.1111/j.1552-6909.2006.00077.x.

Rashid AA, Shamsuddin NH, Ridhuan RDARM, Sallahuddin NA, Devaraj NK. 2018. Breastfeeding practice, support, and self-efficacy among working mothers in a rural health clinic in Selangor. Mal J Med Health Sci 14(2): 39–49.

Shukri NHM, Wells J, Mukhtar F, Lee MHS, Fewtrell M. 2017. Study protocol: An investigation of mother-infant signalling during breastfeeding using a randomised trial to test the effectiveness of breastfeeding relaxation therapy on maternal psychological state, breast milk production and infant behaviour and growth. Int Breastfeed J 12(33):1–14. https://doi.org/10.1186/s13006-017-0124-y.

The Global Economy. 2018. Malaysia: female labor force participation. [Internet]. https://www.theglobaleconomy.com/Malaysia/Female_labor_force_participation/ [Accessed 29th June 2019].

Tokat MA, Okumus H, Dennis CL. 2010. Translation and psychometric assessment of the breast-feeding self efficacy scale-short form among pregnant and posnatal women in Turkey. Midwifery 26(1):101–108. https://doi.org/10.1016/j.midw.2008.04.002.

Utarir AP, Rosita K, Damanik MRM. 2014. Pengetahuan gizi, keluhan kesehatan, kondisi psikologi, dan pola pemberian asi ibu postpartum. Jurnal Gizi dan Pangan 8(3):187–192. https://doi.org/10.25182/jgp.2013.8.3.187-194.

Victora CG, Bahl R, Barros AJD, Franca GVA, Horton S, Kresevec J, Murch S, Sankar MJ, Walker N, Rollins NC. 2016. Breastfeeding in the 21st century: Epidemiology, mechanisms, and lifelong effect. The Lancet 387(10017):475–490. https://doi.org/10.1016/S0140-6736(15)01024-7.

[WHO] World Health Organization. Global targets 2025: To improve maternal, infant and young child nutrition. 2019. http://www.who.

int/nutrition/global-target-2025/en/ [Accessed 25th June 2019].

Yang X, Gao LL, Ip WY, Chan WCS. 2016. Predictors of breast feeding self-efficacy in the immediate postpartum period: A cross-sectional study. Midwifery 41:1–8. https://doi.org/10.1016/j.midw.2016.07.011.

Zhu Y, Zhang Z, Ling Y, Wan H. Impact of intervention on breastfeeding outcomes and determinants based on theory of planned behavior. Women and Birth 30(2): 146–152 https://doi.org/10.1016/j.