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Thyroid Dysfunction, Total Cholesterol Levels and Anthropometric Status in Women of Reproductive Age in Iodine Deficient Area of Prambanan Sub-District, Sleman Regency, Indonesia

M Mutalazimah[1*], Geeta Appannah[2], Nur Lathifah Mardiyati[1], Farida Nur Isnaeni[1], Pramudya Kurnia[1]

[1]Department of Nutrition Science, Faculty of Health Science, Universitas Muhammadiyah Surakarta, Sukoharjo 57102, Indonesia

[2]Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang 43400, Selangor Darul Ehsan, Malaysia

ABSTRACT

This study aimed to determine the relationship between thyroid dysfunction with Total Cholesterol levels (TC), Body Mass Index (BMI), Waist Circumference (WC) and Waist to hip Circumference Ratio (WHR). The cross-sectional observational study was conducted in the endemic iodine deficiency areas in Prambanan Sub-district, Sleman Regency. A total subject of 134 women of reproductive age were selected randomly from the source population of 592. Thyroid dysfunction determined by Thyroid Stimulating Hormone (TSH) and Free Thyroxine (FT4) level were measured by ELISA method. Total cholesterol level was measured by colorimetric method using a spectrophotometer. We also measured the weight and height for BMI calculation as well as the WC and Hip Circumference (HC). Chi-Square test was applied to analyze association between thyroid dysfunction with TC levels, BMI, WC and WHR. Results found the percentage of thyroid dysfunction in subjects was 39.6% (hypothyroidism 4.5% and hyperthyroidism 35.1%). The percentage of subjects with hypercholesterolemia was 34.3%. The BMI calculation found that the underweight, overweight and obesity proportions were 6.7%; 16.4% and 27.6% respectively; WC >80 cm was 29.9% and WHR >0.85 was 38.8%. There was no association between thyroid dysfunction and TC levels, BMI and WC ($p>0.05$) respectively. Meanwhile thyroid dysfunction was significantly associated with WHR ($p<0.05$). Therefore, women in reproductive age with thyroid dysfunction should be aware of their increasing abdominal adiposity.

Keywords: body mass index, thyroid dysfunction, total cholesterol levels, waist circumference, waist to hip circumference ratio

INTRODUCTION

Iodine Deficiency Disorder (IDD) decreases quality of life in all age groups, starting from fetus, neonatal, children, adolescents, adults, and elderly (Eastman & Zimmermann 2018). The negative health impact of iodine deficiency includes a very wide spectrum of problem such as miscarriage, stillbirth, congenital defects, prenatal death, fetal death, cretinism, goiter, hypothyroidism, IQ decrease, mental dysfunction, muscle dysfunction, stunted growth, and Iodine Induced Hyperthyroidism (IIH) (Eastman *et al.* 2019). Several problems related to iodine and thyroid dysfunction occur more likely in women than men (Castello & Caputo 2019). Hence, it is recommended that women do regular screening for iodine deficiency, especially for those who are older than 35 years old. The younger the better, to prepare for pregnancy because failed early detection will results in significant risks on fetal death, congenital hypothyroidism, cretinism, mental retardation, psychomotor developmental disorders, and decreased intelligence in infants (Pearce *et al.* 2016; Eastman & Zimmermann 2018).

One of the indicators used to determine the endemicity of iodine deficiency in a region is using the prevalence of Total Goiter Rate (TGR), which is the total prevalence value of enlarged thyroid glands of a population in a region, either it is a grade I (palpable) or grade II (visible) with the threshold of 5% (WHO 2014). The survey result in Prambanan Sub-district, Sleman

[*]**Corresponding Author:** tel: +62271717417, email: mutalazimah@ums.ac.id

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Regency, Yogyakarta, Indonesia showed that TGR in women was 13%. Thus, defined as an endemic area of iodine deficiency (Mutalazimah *et al.* 2013). Severe iodine deficiency is the cause of hypothyroidism, mild to moderate iodine deficiency have a higher prevalence of hyperthyroidism. Hyperthyroidism that occurs in areas of iodine deficiency is caused by IIH mechanism (Ross *et al.* 2016). Furthermore, some studies found the relation of the effect of thyroid dysfunction towards metabolic syndrome with the indicator of hypercholesterolemia, hyperlipidemia, and obesity (Khatiwada *et al.* 2016; Xu *et al.* 2019).

International Diabetes Federation (IDF) defines metabolic syndrome as a cluster of the most dangerous heart attack risk factors: diabetes and raised fasting plasma glucose, abdominal obesity, high cholesterol and high blood pressure (IDF 2007). Obesity could be interpreted by using anthropometric measurement such as Body Mass Index (BMI) and Waist Circumference (WC), which are used as one of the predictor for metabolic syndrome (Wong *et al.* 2021). Similarly, increasing waist circumference (Ross *et al.* 2020) and Waist to Hip Circumference Ratio (WHR) could represent abdominal adiposity when compared to BMI (Mulyasari & Pontang 2018). Previous study also found that WC and WHR can be used for predicting insulin resistance in adolescents (Fitriyanti *et al.* 2019).

Thyroid dysfunction is commonly defined through Thyroid Stimulating Hormone (TSH) level and Free Thyroxine (FT4) level. Thyroid hormone functions to control metabolism and energy homeostatic and influence body weight, thermogenesis, lipolysis, and cholesterol metabolism. Moreover, TSH serves as the receptor in fatty tissue, induces the differentiation of pre-adipocytes become adipocytes and the expansion of adipose tissue (adipogenesis) (Volke & Krause 2021). Thyroid dysfunction is also strongly related to body weight (Ríos-Prego *et al.* 2019). The increase of TSH level with normal level of thyroid hormone concentration shows subclinical hypothyroidism, and it has been constantly found on obese subjects (Ríos-Prego *et al.* 2019; Mahdavi *et al.* 2021).

Thyroid hormones are essential for women reproductive system, through its roles in the metabolism and growth of ovarian, uterine, and placental tissues. This is related to the interaction of the thyroid hormone with multiple reproductive hormones such as estrogen and prolactin. Thyroid dysfunction in women in of reproductive age can caused menstrual irregularity, endometrial disorder, infertility and the risk of causing pregnancy disorders such as uterine growth disorder, pre-eclampsia, miscarriage and premature birth (Silva *et al.* 2018). Therefore, this research aimed to describe thyroid dysfunction based on TSH and FT4 level on women of reproductive age in iodine deficiency endemic areas, which was in Prambanan Sub-district, Sleman Regency, Yogyakarta, Indonesia. In addition, considering the role of thyroid hormone in human metabolism this study also aimed to determine its relationship with total cholesterol levels and anthropometrics status using BMI, WC and WHR as predictors for metabolic syndrome.

METHODS

Design, location, and time

This cross-sectional study was conducted in Gayamharjo, Wukirharjo and Sumberharjo villages in Prambanan Sub-district, Sleman Regency, Yogyakarta, Indonesia from June to September 2018. This research was approved by ethics committee from Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine Universitas Gadjah Mada Yogyakarta with reference number: KE/FK/270/EC. Each respondent has also signed an informed consent form.

Sampling

The target population in this study were all reproductive age women aged 18−45 years in endemic areas of IDD in Prambanan Sub-district of 3,589 women: the villages of Gayamharjo (n=736), Wukirharjo (n=535) and Sumberharjo (n=2,318). This study excluded subjects without contraception (n=714) and taking hormonal contraception (n=2283), therefore the source population who did not use hormonal contraception was 592 women: Gayamharjo (n=92), Wukirharjo (n=132) and Sumberharjo (n=368).

Sample size was calculated from the source population of 592 women using the following formula, $n=Z\alpha^2.P(1-P)/d^2$ ($Z\alpha$ with confidence level 95%=1.96; P, proportion of

TGR in Prambanan Sub-district=0.13; 1-P=0.87; d, relative precision=0.06). From the Sample Size Version 2.0 software it was computed 121 sample, a 10% non-response rate anticipated was using in this study. Thus, the total sample was 134 women and they were selected using computerized simple random sampling by IBM SPSS for Windows Version 23.0. All of those 134 women were assessed by physicians using checklist questionnaire based on signs and symptoms related to severe endocrine disorder and chronic diseases, such as thyroid cancer, thyrotoxicosis, chronic diabetes mellitus, Grave's diseases, chronic cardiovascular disease, chronic emphysema, chronic kidney failure, chronic liver cirrhosis, and various malignancies. None of them had any of those signs and symptoms, therefore all of those 134 women were eligible to participate in this study.

Data collection

Socio-demographic data (age, education level, occupation, family income) were obtained by interview using a questionnaire. The TC levels was measured by colorimetric method using spectrophotometer. Anthropometric assessments in this study using the BMI, WC and WHR were done by trained nutritionist. BMI calculated as weight in kilograms divided by the square of height in meters (kg/m^2), body weight was measured using digital body scale with precision 0.1 kg (Camry, Indonesia), while height was measured using microtoise stature meter 200 cm with precision 0.1 cm (GEA, Indonesia). The WC and HC was measured using measuring tape and stated in centimeters (cm); and the WHR was calculated as WC divided by the HC. Thyroid dysfunction was interpreted from TSH and FT4 levels using ELISA method, trough collecting 7 ml of venous blood sample by professional medical laboratory staff, which was analyzed in Clinical Pathology Laboratory of dr. Sardjito Hospital Yogyakarta.

Data analysis

In this study TC levels were classified as normal if the value <200 mg/dl and high if the value ≥200 mg/dl (Prihantini 2021). BMI data were classified according to Asian criteria: underweight (<18.5kg/m^2), normal weight (18.5 to <23.0 kg/m^2), overweight (23.0 to <25.0 kg/m^2), obesity (≥25 kg/m^2): obesity I (25–29.9 kg/m^2), and obesity II (≥30 kg/m^2) (Pengpid & Peltzer 2017). BMI data were also categorized into abnormal (underweight, overweight, obesity) and normal (normal weight). The WC cut off point of 80 cm for women to identify risk of Type 2 Diabetes Mellitus (T2DM) among Indonesian population (Harbuwono *et al.* 2020) and the WHR cut off point of 0.85 for women to determine risk of metabolic syndrome (Adegoke *et al.* 2021).

Thyroid function is defined through the measurement result of Thyroid Stimulating Hormone (TSH) and Free Thyroxine (FT4). Conventional reference intervals for TSH and FT4 were 0.4−4.0 mIU/l (Razvi *et al.* 2020) and 0.8–1.8 ng/dl (Aubert *et al.* 2017), respectively. Based on the reference interval, thyroid function is divided into two groups, namely the thyroid dysfunction group and the normal thyroid function group. Thyroid dysfunction was defined as: (1) subclinical hypothyroidism, if TSH level above reference interval, with FT4 was still in the normal reference range; (2) clinical hypothyroidism was indicated by the increase of TSH and the decrease of FT4; (3) subclinical hyperthyroidism if TSH level below reference value, with FT4 was still in the normal reference interval; (4) clinical hyperthyroidism was indicated by the decrease of TSH and the increase of FT4. Meanwhile the normal thyroid function if both TSH and FT4 were still in the normal reference interval (Arce-Sánchez *et al.* 2021). In this study, thyroid dysfunction was classified as "Yes" (for subjects with subclinical hypothyroidism, clinical hypothyroidism, subclinical hyperthyroidism, and clinical hyperthyroidism) and "No" (for subject with normal thyroid function).

IBM SPSS Statistic for Windows Version 23.0 (IBM Corp, Armonk, New York, US) was used to process and analyze data. Univariate analysis was carried out by describing all categorical data in terms of frequency and percentage, while numerical data was described by presenting mean and Standard Deviation (SD) for normal distribution data, and presenting median and range for non-normal distribution data. Numerical data analyzed for normality were TSH, FT4, total cholesterol, BMI, WC and WHR. TSH was not normally distributed, therefore descriptive analysis used the median and range, meanwhile other data that were normally distributed described using the mean and SD. Association of thyroid dysfunction, total

cholesterol levels and anthropometry (BMI, WC and WHR) was analyzed using Chi-Square test. A threshold of $p<0.05$ was set as significance level.

RESULTS AND DISCUSSION

Socio-demographic characteristics of subjects

Socio-demographic data described and analyzed were age, educational level, occupation, and family income (Table 1).

Table 1 shows that this study found the percentage of women who were at risk for reproductive problem based on age was 37.3%. Overall, the educational level of respondents was still relatively low, namely 3.0% illiterate and 25.4% with elementary school. This study, which was conducted in the mountainous area, found that most women were mostly housewife (51.5%) and the second most common occupation was farmer (26.9%). This study found 90.3% of subjects had a monthly family income below the Sleman Regency regional minimum wage in 2018 (IDR 1,574,550.00 or $109).

Thyroid function in subjects

The interpretation of TSH and FT4 level as the indicator of thyroid dysfunction defined by normal reference interval of 0.4−4.0 mIU/l and 0.8−1.8 ng/dl, respectively. The median (range) for TSH was 0.59 (0.004−22.4) mIU/l and the mean FT4 level was 0.99±0.27 ng/dl. For the thyroid function categorization, TSH could not stand alone, it needs to be interpreted simultaneously with FT4 level. The description of the thyroid function in subjects are presented in Table 2.

We found 39.6% of all subjects had thyroid dysfunction, where 35.1% was in the form of hyperthyroidism and 4.5% suffered from hypothyroidism. (Table 2). Previous study in Magelang Regency, Central Java found

Table 1. Socio-demographic characteristics of subjects

Variables	n (%)
Age (year)	
At risk from reproductive problems (<20 or >35)	50 (37.3)
Not at risk from reproductive problems (20−35)	84 (62.7)
Mean±SD of age (year)	33.81±7.09
Education	
Unschooling	4 (3.0)
Elementary school	34 (25.4)
Junior high school	44 (32.8)
Senior high school	45 (33.6)
University	7 (5.2)
Occupation	
Housewife	69 (51.5)
Farmer	34 (26.9)
Merchant	2 (1.5)
Private employee	24 (17.9)
Government employee	3 (2.2)
Family income (IDR)	
<Minimum regional wage (1,574,550.00)	121 (90.3)
≥Minimum regional wage (1,574,550.00)	13 (9.7)
Mean±SD of family income (IDR)	729,477±456,828

IDR: Indonesian Rupiah; SD: Standart Deviation

Table 2. Thyroid function in subjects

Variables	n (%)
Thyroid function	
Thyroid dysfunction	53 (39.6)
Subclinical hypothyroidism	2 (1.5)
Clinical hypothyroidism	4 (3.0)
Subclinical hyperthyroidism	45 (33.6)
Clinical hyperthyroidism	2 (1.5)
Normal thyroid function	81 (60.4)
Thyroid dysfunction status	
Yes	53 (39.6)
No	81 (60.4)
TSH level (mIU/l)	
Median (range) of TSH level (mIU/l)	0.59 (0.004-22.4)
FT4 level (ng/dl)	
Mean±SD of FT4 level (ng/dl)	0.99±0.27

TSH: Thyroid Stimulating Hormone; FT4: Free Thyroxine; SD: Standart Deviation

lightly higher prevalence which was 39.8% (Nurcahyani *et al.* 2020). The higher prevalence of hyperthyroidism than hypothyroidism in IDD endemic areas might be caused by Iodine Induced Hyperthyroidism (IIH). This often associated with the use of iodine prophylaxis such as excess of iodine supplementation, high iodine-containing drugs consumption and frequent consumption of iodine fortified food (Mutalazimah *et al.* 2013). The percentage of clinical hypothyroidism and hyperthyroidism in this study were similar the epidemiology report, which found that the prevalence of clinical hypothyroidism was approximately 1–2%. Similarly, it was reported that the prevalence of clinical hyperthyroidism was 0.5–2%. Meanwhile, the percentage of subclinical hypothyroidism in this study was lower than the findings of National Health and Nutrition Examination Survey (NHANES III), ranging between 4 and 21%. However, the percentage of subclinical hyperthyroidism in this study was higher than the results of a mini review in some surveys conducted in Indonesia, which found that hyperthyroidism was 6.9% (Miharja & Karyana 2019).

Hyperthyroidism was often found in IDD endemic areas this could happen through the mechanism of Autonomous Functioning Thyroid Nodule (AFTN). This mechanism is a biological fundamental reason for hyperthyroidism, often caused by mutation of thyroid cells which causes the autonomous function. Another cause of hyperthyroidism on subjects with iodine deficiency is family history of autoimmune condition, inherited from parents through thyroid-specific genes mechanism, similar to TSH receptor (TSHr) and Thyroglobulin (Tg), which loses its responsibility and causing unwarranted feedback mechanism disorders of the thyroid hormone (Stefan & Faustino 2017). Even though most of them are considered subclinical, it is possible to develop into overt or secondary disorder phase, if it is not anticipated with adequate intervention.

Total cholesterol levels, BMI, WC and WHR in subjects

The mean of total cholesterol levels, WC and WHR was still in normal category. Meanwhile, the mean BMI was in the overweight category. The mean/median and category of total cholesterol levels, BMI, WC, and WHR are presented Table 3.

This study found that more than one third (34.3%) of subjects had total cholesterol

Table 3. Total cholesterol levels and anthropometric status (BMI, WC, and WHR) in subjects

Variables	n (%)
Total Cholesterol levels (mg/dl)	
Hypercholesterolemia (>200)	46 (34.3)
Normal (≤200)	88 (65.7)
Mean±SD of total cholesterol (mg/dl)	177.21±39.58
BMI	
Underweight (<18.5)	9 (6.7)
Normal (18.5−22.9)	66 (49.3)
Overweight (23.0−24.9)	22 (16.4)
Class I Obesity (25.0−29.9)	26 (19.4)
Class II Obesity (>30.0)	11 (8.2)
Mean±SD of BMI	23.40±3.99
WC (cm)	
At risk from abdominal obesity (>80)	40 (29.9)
Not at risk from abdominal obesity (≤80)	94 (70.1)
Mean±SD of WC (cm)	75.92±8.88
WHR	
At risk from abdominal obesity (>0.85)	52 (38.8)
Not at risk from abdominal obesity (≤0.85)	82 (61.2)
Mean±SD of WHR	0.82±0.06

BMI: Body Mass Index; WC: Waist Circumference; WHR: Waist to Hip Ratio; SD: Standard Deviation

levels of >200 mg/dl or suffered from hypercholesterolemia; this is an increasing concern considering the fact that the subjects lived in rural mountainous area. It was assumed that they were not exposed to the risk factors of hypercholesterolemia such as fast food or food with high cholesterol level. This prevalence was higher compared to a study conducted by Nurcahyani *et al.* (2020) in IDD clinic of research and development center Magelang, Central Java, Indonesia which was 21.7%. This study found 44.0% of subjects were overweight or obese and 6.7% was underweight. This was higher than the previous study which was 38.6% (Nurcahyani *et al.* 2020). We found 38.8% of subjects with WHR of >0.85, which was included in the category of at risk towards abdominal adiposity. Hence, this study add to the evidence that the risk of obesity did not only threaten to modern community in urban areas, but also to the rural community in mountainous areas. However, the prevalence was lower than a previous study in Iran which found that 68.67% of women with WHR of >0.85 (Shahvazi *et al.* 2017).

Association of thyroid dysfunction, total cholesterol levels and anthropometric status (BMI, WC and WHR)

The association between thyroid dysfunction and total cholesterol levels, BMI, WC and WHR were tested using Chi-Square test as shown in Table 4.

Table 4. Association of thyroid dysfunction, TC, BMI, WC, and WHR

	Thyroid dysfunction	Potential risks		p
		n	%	
Hypercholesterolemia	Yes	16	30.2	0.414
	No	30	37.0	
Non hypercholesterolemia	Yes	37	69.2	
	No	51	63.0	
BMI abnormal	Yes	30	56.6	0.273
	No	38	46.9	
BMI normal	Yes	23	43.4	
	No	43	53.1	
WC at risk	Yes	18	34.0	0.400
	No	22	27.2	
WC not at risk	Yes	35	66.0	
	No	59	72.8	
WHR at risk	Yes	15	28.3	0.044
	No	37	45.7	
WHR not at risk	Yes	38	71.7	
	No	44	54.3	

BMI: Body Mass Index; TC: Total Cholesterol Level; WC: Waist Circumference; WHR: Waist to Hip Ratio

Chi-Square test showed that there was no significant relationship between thyroid dysfunction, TC, BMI and WC (p=0.414; p=0273; p=0.400) respectively. The findings were in line with previous studies conducted in China, in which they found no significant relation between TSH and total cholesterol, as well as TSH and BMI (Xu *et al.* 2019). The most fundamental factor that determined the relevance with previous studies was the existence of other factors as driving factors in the occurrence of metabolic syndrome, which were not studied in this research such as age, dietary pattern, and activity pattern. There was also another determining factor in a study conducted in China with samples that were predetermined as patients with Coronary Heart Disease (CHD); whereas, in this research, the metabolic syndrome condition of each sample was not known in advance. The previous study discovered there is a significant relationship between TSH changes and alterations of WC in women in Iran (Motamed *et al.* 2016).

Our study found that thyroid dysfunction was associated with WHR (p=0.044). TSH through receptor in fatty tissue induced the differentiation of pre-adipocytes to become adipocytes and the expansion of adipose tissue (adipogenesis). The increase of TSH level with normal concentration level of thyroid hormone showed subclinical hypothyroidism had been consistently found in obese subjects. The production of TSH was also regulated by neurotransmitter and hormone that affected body weight such as neuro peptide and alpha melanocyte stimulating hormone that were bound by peptides and connected with hypophysiotropic hormone namely Thyrotropin Releasing Hormone (TRH) neurons. The neurotransmitter and hormone were also affected by leptin. TSH also directly induced the synthesis and release of adipokines, in which some of the

adipokines functioned to control appetite through regulatory mechanisms in the brain (Volke & Krause 2021). Hence, TSH is related to the pattern of appetite and induces adipocyte tissue; therefore, it is related to the measurable weight changes reflected in WHR.

CONCLUSION

This study found clinical and subclinical thyroid dysfunction, both hypothyroidism and hyperthyroidism in women of reproductive age living in IDD endemic area. Thyroid dysfunction had significance association to WHR. Thus, awareness of nutritional status, particularly WHR indicator is important for this demography. WHR reflects the accumulation of abdominal adiposity, thus efforts to reduce the risk of high WHR in women reproductive age with thyroid dysfunction should include maintaining a balanced diet and doing regular physical activity. Follow up studies with better design such as case control or cohort approach are pivotal. It is also important to utilized a more complete markers for metabolic syndrome which include all lipid profiles (LDL, HDL, and triglyceride).

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DECLARATION OF INTERESTS

The authors confirm that there are no known conflicts of interest related with this paper. All authors have made substantial contributions in the study and manuscript writing.

REFERENCES

Adegoke O, Ozoh OB, Odeniyi IA, Bello BT, Akinkugbe AO, Ojo OO, Agabi OP, Okubadejo NU. 2021. Prevalence of obesity and an interrogation of the correlation between anthropometric indices and blood pressures in urban Lagos, Nigeria. Sci Rep 11(1):1−12. https://doi.org/10.1038/s41598-021-83055-w

Arce-Sánchez L, Vitale SG, Flores-Robles CM, Godines-Enriquez MS, Noventa M, Urquia-Figueroa CM, Martínez-Cruz N, Estrada-Gutierrez G, Sosa SEY, Romo-Yañez J, *et al.* 2021. Effect of the cut-off level for thyroid-stimulating hormone on the prevalence of subclinical hypothyroidism among infertile Mexican women. Diagnostics 11(3):417. https://doi.org/10.3390/diagnostics11030417

Aubert CE, Floriani C, Bauer DC, Da Costa BR, Segna D, Blum MR, Collet TH, Fink HA, Cappola AR, Syrogiannaoli L, *et al.* 2017. Thyroid function tests in the reference range and fracture: Individual participant analysis of prospective cohorts. J Clin Endocrinol Metab 102(8):2719−2728. https://doi.org/10.1210/jc.2017-00294

Castello R, Caputo M. 2019. Thyroid diseases and gender. Ital J Gend-Specif Med 5(3):136−141. https://doi.org/10.1723/3245.32148

Eastman CJ, Zimmermann MB. 2018. The iodine deficiency disorder. https://www.ncbi.nlm.nih.gov/books/NBK285556/ [Accessed 27th September 2021].

Eastman CJ, Ma G, Li M. 2019. Optimal assessment and quantification of iodine nutrition in pregnancy and lactation: Laboratory and clinical methods, controversies and future directions. Nutrients 11(10):2378. https://doi.org/10.1177/089033448700300322

Fitriyanti AR, Sulchan M, Tjahjono K, Sunarto S. 2019. Prediction of insulin resistance in late adolescent based on anthropometric index. J Gizi Pangan 14(2):53−60. https://doi.org/10.25182/jgp.2019.14.2.53-60

Harbuwono DS, Tahapary DL, Tarigan, TJE, Yunir E. 2020. New proposed cut-off of waist circumference for central obesity as risk factor for diabetes mellitus: Evidence from the Indonesian basic national health survey. Plos One 15(11):e0242417. https://doi.org/10.1371/journal.pone.0242417

[IDF] International Diabetes Federation. 2007. IDF Consensus Worldwide Definition of the Metabolic Syndrome in Children and Adolescents. Belgium (BE): IDF.

Khatiwada S, Sah SK, KC R, Baral N, Lamsal M. 2016. Thyroid dysfunction in metabolic syndrome patients and its relationship with

components of metabolic syndrome. Clin Diabetes Endocrinol 2(1):1−5. https://doi.org/10.1186/s40842-016-0021-0

Mahdavi M, Amouzegar A, Mehran L, Madreseh E, Tohidi M, Azizi F. 2021. Investigating the prevalence of primary thyroid dysfunction in obese and overweight individuals: Tehran thyroid study. BMC Endocr Disord 21(1):1−12. https://doi.org/10.1186/s12902-021-00743-4

Miharja L, Karyana M. 2019. Hyperthyroidism in Indonesian community: A mini review. Endocrinology Metab Int J 7(2):52−53. https://doi.org/10.15406/emij.2019.07.00241

Motamed B, Eftekharzadeh A, Hosseinpanah F, Tohidi M, Hasheminia M, Azizi F. 2016. The relation between changes in thyroid function and anthropometric indices during long-term follow-up of euthyroid subjects: The Tehran thyroid study (TTS). Eur J Endocrinol 175(4):247−253. https://doi.org/10.1530/EJE-16-0414

Mulyasari I, Pontang GS. 2018. Waist circumference and waist-to-height ratio as indicators for excess adiposity in adolescents. J Gizi Pangan 13(3):131−136. https://doi.org/10.25182/jgp.2018.13.3.131-136

Mutalazimah M, Mulyono B, Murti B, Azwar S. 2013. Asupan Yodium, ekskresi yodium urine, dan goiter pada wanita usia subur di daerah endemis defisiensi yodium. Kesmas: Jurnal Kesehatan Masyarakat Nasional 8(3):133−138. https://doi.org/10.21109/kesmas.v8i3.359

Nurcahyani YD, Sukandar PB, Fitriyanto RE, Akhmad SA. 2020. Relationship between thyroid hormone and lipid profile in patients with struma at the iodine deficiency disorder (IDD) clinic of research and development center Magelang. Jurnal Kedokteran dan Kesehatan Indonesia 11(2):130−140. https://doi.org/10.20885/JKKI.Vol11.Iss2.art5

Pearce EN, Lazarus JH, Moreno-Reyes R, Zimmermann MB. 2016. Consequences of iodine deficiency and excess in pregnant women: An overview of current knowns and unknowns. Am J Clin Nutr 104:918S−923S. https://doi.org/10.3945/ajcn.115.110429

Pengpid S, Peltzer K. 2017. The prevalence of underweight, overweight/obesity and their related lifestyle factors in Indonesia, 2014−15. AIMS Public Health 4(6):633−649. https://doi.org/10.3934/publichealth.2017.6.633

Prihantini NN. 2021. Correlation between dieting patterns with total cholesterol levels in blood in the hkbp church congregation on September 2018. Int J Res Granthaalayah 9(4):240−255. https://doi.org/10.29121/granthaalayah.v9.i4.2021.3864

Razvi S, Ryan V, Ingoe L, Pearce SH, Wilkes S. 2020. Age-related serum thyroid-stimulating hormone reference range in older patients treated with levothyroxine: A randomized controlled feasibility trial (SORTED 1). Eur Thyroid J 9(1):40−48. https://doi.org/10.1159/000504047

Ríos-Prego M, Anibarro L, Sánchez-Sobrino P. 2019. Relationship between thyroid dysfunction and body weight: A not so evident paradigm. Int J Gen Med 12:299−304. https://doi.org/10.2147/IJGM.S206983

Ross DS, Burch HB, Cooper DS, Greenlee MC, Laurberg P, Maia AL, Rivkees SA, Samuels M, Sosa JA, Stan MN, *et al.* 2016. 2016 American thyroid association guidelines for diagnosis and management of hyperthyroidism and other causes of thyrotoxicosis. Thyroid 26(10):1343−1421. https://doi.org/10.1089/thy.2016.0229

Ross R, Neeland IJ, Yamashita S, Shai I, Seidell J, Magni P, Santos RD, Arsenault B, Cuevas A, Hu FB, *et al.* 2020. Waist circumference as a vital sign in clinical practice: A Consensus Statement from the IAS and ICCR Working Group on Visceral Obesity. Nat Rev Endocrinol 16(3):177−189. https://doi.org/10.1038/s41574-019-0310-7

Shahvazi S, Nadjarzadeh A, Mehri Z, Salehi-Abarguoei A. 2017. Comparison between different criteria to estimate obesity prevalence and its correlates in female adults residing in central Iran. Prog Nutr 19(3):295−304. https://doi.org/10.23751/pn.v19i3.5175

Silva JS, Ocarino NM, Serakides R. 2018. Thyroid hormones and female reproduction.

Biol Reprod 99(5):907−921. https://doi.org/10.1093/biolre/ioy115

Stefan M, Faustino LC. 2017. Genetics of thyroid-stimulating hormone receptor-relevance for autoimmune thyroid disease. Front Endocrinol 8:57. https://doi.org/10.3389/fendo.2017.00057

Volke L, Krause K. 2021. Effect of thyroid hormones on adipose tissue flexibility. Eur Thyroid J 10(1):1−9. https://doi.org/10.1159/000508483

[WHO] World Health Organization. 2014. Goitre as a Determinant of the Prevalence and Severity of Iodine Deficiency Disorders in Populations. Geneva (CH): WHO.

Wong JC, O'Neill S, Beck BR, Forwood MR, Khoo SK. 2021. Comparison of obesity and metabolic syndrome prevalence using fat mass index, body mass index and percentage body fat. Plos One 16:e0245436. https://doi.org/10.1371/journal.pone.0245436

Xu R, Huang F, Zhang S, Lv Y, Liu Q. 2019. Thyroid function, body mass index, and metabolic risk markers in euthyroid adults: A cohort study. BMC Endocr Disord 19(1):1−9. https://doi.org/10.1186/s12902-019-0383-2

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Validity and Reproducibility of Malaysian Food Frequency Questionnaire for Dietary Intake Related to Colorectal Cancer

Ainaa Almardhiyah Abd Rashid[1], Lydiatul Shima Ashari[1], Nor Hamizah Shafiee[2], Raja Affendi Raja Ali[3,4], Yeong Yeh Lee[4,5,6], Mohd Razif Shahril[7], Hamid Jan Jan Mohamed[1*]

[1]Nutrition Programme, School of Health Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia
[2]Department of Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, 56000 Kuala Lumpur, Malaysia
[3]Gastroenterology Unit, Department of Medicine, UKM Medical Centre, 56000 Kuala Lumpur, Malaysia
[4]Gut research group, Faculty of Medicine, Universiti Kebangsaan Malaysia, 56000 Kuala Lumpur, Malaysia
[5]School of Medical Sciences, Universiti Sains Malaysia, 16150 Kota Bharu, Kelantan, Malaysia
[6]GI Function & Motility Unit, Hospital USM, Universiti Sains Malaysia, 16150 Kota Bharu, Kelantan, Malaysia
[7]Nutritional Science Programme and Centre for Healthy Ageing and Wellness (HCARE), Faculty of Health Sciences, Universiti Kebangsaan Malaysia, 50300 Kuala Lumpur, Malaysia

ABSTRACT

This study aims to report on the validity and reproducibility of a 142-food item Food Frequency Questionnaire (FFQ) for dietary factors related to colorectal cancer among Malaysians. Population aged 30 to 70 years from two cities of Peninsular Malaysia were recruited through voluntary participation. A semi-quantitative FFQ was modified from an established FFQ used in the national survey. It includes specific questions to measure the consumption of food sources related to colorectal cancer development. FFQ was administered two times in two weeks to evaluate reproducibility (FFQ1 and FFQ2). Then the validity was assessed by comparing FFQ against the 3-day Food Record method (FR). A total of 100 respondents (mean age 50.6 years) provided data for both validity and reproducibility. The FFQ had significantly higher estimates of most nutrients and food groups' intake than the FR. The Spearman correlation showed moderate agreement between FFQ and FR while moderate to strong correlation between FFQs. The limit of agreement between both methods using Bland Altman plot was acceptable for both validity and reproducibility. The classification into the same and adjacent quartiles was between 62 to 75% for validity and 77 to 89% for reproducibility assessment. Overall, the validity was satisfactory and reproducibility of the FFQ was good for estimating absolute nutrient and food group intakes. Hence, the FFQ could be used as a valid tool for assessing dietary intake among Malaysians to study dietary factors related to colorectal cancer risk.

Keywords: colorectal cancer, food frequency questionnaire, food record, reproducibility, validation

INTRODUCTION

Globally, the estimated number of incident cases of Colorectal Cancer (CRC) is around 1,9 million and for death cases are 935,000. This estimation ranked CRC as fourth and second in terms of incidence and mortality respectively among other cancer sites. The highest rate of colon cancer incidence was found in European regions, Australia/New Zealand, and Northern America while for rectal cancer the rate of distribution among those regions is similar including Eastern Asia (International Agency for Research on Cancer 2020). The trend of CRC incidence is increasing, the incidence of colon cancer was found to increase in 10 out of 36 countries from 2007 to 2016, 2006 to 2015 or 2005 to 2014 where India dominated the highest increment followed by Poland (International Agency for Research on Cancer 2020). In Malaysia, the estimated number of new cases of colon and rectum cancer is around 3,816 and 2,690 respectively while the death cases of colon and rectum cancer are 2,035 and 1,385 respectively (International Agency for

[*]**Corresponding Author:** tel: +6097677618, email: hamidjan@usm.my
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Research on Cancer 2020). A study by Ibrahim and colleagues (Ibrahim *et al*. 2020) reported that the age-standardised incidence and mortality trends from 2007 to 2017 for patients under 50 years of age were stable but this group were found to constitute a considerable proportion of CRC in northern Malaysia.

There are various risk factors of CRC namely non-modifiable and modifiable risk factors. Non-modifiable risk factors comprising race and ethnicity, sex, age, hereditary mutations, inflammatory bowel disease, abdominal radiation, cystic fibrosis, cholecystectomy, and androgen deprivation therapy while modifiable risk factors including obesity and physical inactivity, diet, smoking, alcohol, medications, and diabetes and insulin resistance (Rawla *et al.* 2019). Single foods and nutrients and dietary patterns become a significant determinant either to increased or decreased CRC risks. A meta-analysis study conducted by McNabb and colleagues (McNabb *et al.* 2020) showed that heavier drinker of alcohol increased CRC risks while another systematic review and meta-analysis study by Morze and colleagues (Morze *et al.* 2021) observed that Mediterranean dietary pattern reduced the cancer mortality in general population and all-cause mortality among cancer survivors as well as colorectal, head and neck, respiratory, gastric, liver and bladder cancer risks.

There are several methods often utilized in epidemiological study for example, Food Records (FR) and 24-hour recalls for current intake assessment and dietary history and Food Frequency Questionnaire (FFQ) for usual or habitual intake assessment (Ocké *et al.* 2020). All these methods had its own strength and limitation and the selection of the method are depending on the purpose and target group of the study (Ocké *et al.* 2020). In investigating the relationship between diets with CRC in Malaysia, FFQ is found to be the most appropriate tool to evaluate the population's habitual or long-term consumption (El Kinany *et al.* 2018). In fact, this tool is cost effective and time-saving. Before the FFQ could be utilised, the validity and reproducibility study should be first conducted. A validation study will examine how accurately the dietary tools measures the true intakes while a reproducibility study will examine the variation in measurements made on respondent over a period of time (Ocké *et al.* 2020). Both validity and reproducibility study on FFQ related to CRC had been conducted earlier among Belgians (Tollosa *et al.* 2017), Dutch (Koole *et al.* 2020), Norwegians (Henriksen *et al.* 2018), and Canadians (Liu *et al.* 2013). In Malaysia, the inadequacy of food items of FFQ from NHMS 2014 to measure CRC has led to FFQ modification. To date there is no validity and reproducibility study on FFQ related to CRC conducted among Malaysian and a study should be carried out as each population has different food supply and dietary habits. Therefore, the study aimed to evaluate the validity and reproducibility of FFQ which includes all colorectal cancer dietary factors in Malaysian adults.

METHODS

Design, location, and time

All respondents voluntarily participated in this study from March to June 2020 and were recruited from Kuala Lumpur (cities of Peninsular Malaysia) and Kota Bharu, Kelantan representing urban and suburban areas respectively. Due to good cooperation, respondents were conveniently recruited. Ethical clearance for the study was granted on 3rd March 2020 by the Human Research Ethics Committee of Universiti Sains Malaysia (USM/JEPeM/19060354).

Sampling

The inclusion criteria for the selection were Malaysian, aged 30 to 70, and did not practice any diet regime. No exclusion of respondents out of 100 respondents into the validation and reproducibility study based on energy intake of 500 to 3,500 kcal/day (van Dongen *et al.* 2019; Willett 2012). According to Cade (2004), the sample size recommended for validation study was 50 to 100 respondents for each demographic group.

The respondents were informed on the objective and methodology of the study and those who agreed to participate were requested to sign the consent form. The dietary information of respondents was assessed using semi-quantitative FFQ1 and the same information was collected again after two weeks using semi-quantitative FFQ2. The three-day Food Record (FR) was distributed to respondents after completion of semi-quantitative FFQ2 in order to avoid bias introduced by increased awareness when completing FR.

Data collection

The semi-quantitative FFQ applied in the study is basically a modification from the National Health and Morbidity Survey (NHMS) 2014. The FFQ from NHMS 2014 consist of 165 food items and several of foods and drinks were excluded at the initial step of the new FFQ development. Forty-one food items were excluded on the basis of rarely consumed by the population and 22 foods or drinks were combined into 1 food item due to its similarities in food group such as white meats, fruit vegetables, legumes, flavours, bread spread, fruits, local fruits, and drinks. Six high risk and protective foods and drinks against CRC was added to FFQ by identifying it from the previous literature and cancer report of continuous update project (World Cancer Research Fund 2018). About 34 food items in FFQ from NHMS 2014 had high risk and protective value to be retained in our present FFQ while remaining 102 items were maintained as frequently eaten by Malaysians. High risk foods were foods that containing high fat (red and processed meat), heme-iron (meat/ poultry/certain fish), nitrite/nitrate (processed meat), and cooking method (baked/ grilled/ deep fried). Protective foods were foods containing omega-3 fatty acids (nut, legumes, fish, and seafood), vitamin D (mushrooms and barley drink), calcium (milk), and fibre (fruits and vegetables). Face and content validity were conducted by two nutritionists to verify the food list and added prominent food to the list. A total number of 142 food items were finalized as the food items list in the FFQ. During data collection, the respondents were requested to recall the intake frequency of foods and drinks and the intake amount for the past one year. The intake frequency provides four options including per day, per weeks, per months, and per year/never. FFQ1 and FFQ2 was administered once each in a period of two weeks.

Three-day FR was carried out to examine the validity of semi-quantitative FFQ by comparing the foods and nutrient intakes between both assessment methods. The respondents were requested to record their diet on three non-consecutive days (2 weekdays and 1 weekend) on type of food and beverages, time of meals, place of eating, and price for take-away foods (Luftimas *et al.* 2021). This is done to capture the variation in meal consumption of the respondents. Photographs of household measurements including glass, cup, tablespoon, teaspoon, etc were provided to aid respondents in estimating the portion sizes of the foods that they consumed.

Data analysis

All the dietary information from the semi quantitative FFQ and three-day FR were analysed using Nutritionist Pro™ Diet Analysis Software version 7.8.0 (Axxya Systems, version 2020, Redmond, USA).

Nutrients and food group's analysis. The Nutrient Composition of Malaysian Foods reference list in the Nutritionist ProTM database was used to select a total of 142 food items from FFQ. To obtain the energy and nutrients values, the daily intake of each food item was entered by calculating using the following formula: frequency of intake per day x total number of servings x weight of food in one serving. For three-day FR, the energy and nutrient values was obtained straight away by selecting the foods and recipes from the reference list of Nutrient Composition of Malaysian Foods. Recipes which were not available in the reference list were added into the database where the portion sizes were calculated based on standard recipe sizes for example total serving and per serving size. Weight of foods or ingredients to make the recipes were referred from the Atlas of Food Exchanges & Portion Sizes, Nutrient Composition of Malaysian Foods, Malaysian Food Album (IPH 2011) and Malaysian Food Composition Database (MyFCD 2020). Nutritional content of the food product was obtained from its packaging or MyFCD and was inserted into the database.

The following food groups were allocated to each of the 142 food items: cereal products, meats, fish and seafoods, eggs, vegetables, legumes, bread spreads, fruits, confectionaries, fast foods, non-sugary drinks, sugar sweetened drinks, alcoholic drinks, condiments and dairy products. In order to obtain the food group intake value from the FFQ, the daily gram intake of food items was summed up according to its food group category. For FR, the total gram intake of foods and meals listed was summed up and divided by 3 days according to its food group.

Reproducibility analysis. Nutrients and food groups' data for FFQ1 and FFQ2 were checked for normality. The mean and 5^{th} and 95^{th} percentiles of nutrients and food groups were calculated for FFQ1 and FFQ2. Comparison

of energy and nutrient intakes between FFQ1 and FFQ2 were analysed using Wilcoxon's sign rank test while relationship between FFQs were determined using Spearman correlation (absolute values and energy-adjusted values). The reproducibility of absolute nutrient and food intakes from the both methods were further assessed by cross-classification analysis. All statistical analysis was performed using IBM SPSS Statistics, Version 26.0 (Chicago, IL, USA). $p<0.05$ was considered as statistically significant.

Validity analysis. Nutrients and food groups' data for FFQ1 and FR were checked for normality. The mean and 5th and 95th percentiles of nutrients and food groups were computed for both assessment methods of FFQ1 and FR. Differences of energy and nutrient intakes between FFQ1 and FR were analysed using paired t-test or Wilcoxon's sign rank test. Spearman or Pearson correlation were used to calculate the strength of the relationship between the two methods. Residual method was used to calculate energy-adjusted values from the total energy intake (Willett 2012). Cross-classification analysis was performed to indicate the potency of the FFQ when matched up with FR to classify individuals into the same or within one quartile of the nutrient and food groups. The agreements between FFQ1 and FR was further assess using Bland-Altman plot and limit of agreement (LOA; ±2SD) was used to define it.

RESULTS AND DISCUSSION

Respondents' characteristics

A total of 100 respondents participated in the reproducibility and validation studies (Table 1). Respondents were recruited from urban (50%) and suburban (50%) area to represent the whole population in Malaysia from different socio-economic background. Mean age of study respondents was 50.6 years; 80% of the respondents aged more than 50 years were enrolled to represent the CRC patients because the CRC incidence increased after the age of 60 years (National Cancer Institute 2019). Meanwhile respondents aged above 30 years represent the cancer patients as the cancer

Table 1. Sociodemographic characteristics of study respondents (n=100)*

Characteristics	Total (n=100) n (%)	Urban (n=50) n (%)	Suburban (n=50) n (%)
Age groups (years) mean (SD)	50.6 (10.1)	52.3 (3.5)	49.0 (13.8)
30−50	20 (20.0)	0 (0.0)	20 (40.0)
50−70	80 (80.0)	50 (100)	30 (60.0)
Race			
Malay	70 (70.0)	20 (40.0)	50 (100.0)
Chinese	17 (17.0)	17 (34.0)	0 (0.0)
Indian	13 (13.0)	13 (26.0)	0 (0.0)
Educational level			
Primary school	10 (10.0)	6 (12.0)	4 (8.0)
Secondary school	51 (51.0)	32 (64.0)	19 (38.0)
University	38 (38.0)	12 (24.0)	26 (52.0)
Unschooling	1 (1.0)	0 (0.0)	1 (2.0)
Household number			
1−5	81 (81.8)	49 (98.0)	32 (65.3)
6−10	13 (13.1)	1 (2.0)	12 (24.5)
≥11	5 (5.1)	0 (0.0)	5 (10.2)
Income classification by household**			
B40	88 (88.0)	48 (96.0)	40 (80.0)
M40	12 (12.0)	2 (4.0)	10 (20.0)
T20	0 (0.0)	0 (0.0)	0 (0.0)

[a]Sample size was not always n=100 due to missing values because one respondent did not answer on the household number

[b]Source: Household Income and Basic Amenities Survey Report 2019; Department of Statistics Malaysia: B40-income less than RM 4,849 (1,158.39 USD); M40-income range RM 4,850 to RM 10,959 (1,158.62 to 2,618.01 USD); T20-income more than RM 10,960 (2,618.25 USD)

incidence increased after the age of 30 years (National Cancer Institute 2019).

There was zero respondent withdrawal as the completeness of three dietary assessments were achieved. Hundred sample size was adequate to determine questionnaire's validity (Willett 2012). The study did not exclude any respondents on the basis of under- and over-reporting dietary intake less than 500 kcal and more than 3,500 kcal (van Dongen *et al.* 2019; Willet 2012). Misreporting has been avoided by energy adjustment approach via residual method instead of respondent exclusion (Liu *et al*. 2013).

Reproducibility

Reproducibility of the FFQ was generated to establish the potency of the FFQ to evaluate nutrient and food group intakes two weeks apart. The FFQ had acceptable reproducibility two weeks apart, with correlation values more than 0.40 for the majority of nutrients (Cade *et al.* 2004). Table 2 present the data analysis between

Table 2. Reproducibility of nutrient and food group between FFQ1 and FFQ2 (n=100)

Energy, nutrients, and food groups	FFQ1 (n=100)	FFQ2 (n=100)	p[a]	Spearman correlation		Cross-classification into quartiles (%)	
	Mean (P_5, P_{95})	Mean (P_5, P_{95})		Unadjusted	Energy adjusted	Correctly classified	Grossly misclassified
Energy (kcal)	2,352 (1,338, 3,393)	2,399 (1,103, 3,182)	0.020	0.60[b]	-	84	2
Protein (g)	97.2 (54.8, 145.7)	106.4 (44.9, 156.1)	0.006	0.64[b]	0.15	88	4
Carbohydrate (g)	312.6 (191.5, 460.6)	328.8 (169.2, 449.0)	0.009	0.45[b]	0.42[b]	80	8
Fat (g)	75.7 (38.8, 119.0)	75.0 (27.5, 118.2)	0.145	0.58[b]	0.45[bc]	89	2
Saturated fat (g)	12.8 (6.0, 22.1)	13.5 (5.0, 25.4)	0.010	0.53[b]	0.38[b]	81	4
Monounsaturated fatty acids (g)	12.4 (6.2, 21.4)	13.1 (4.8, 21.5)	0.006	0.51[b]	0.33[b]	81	3
Polyunsaturated fatty acids (g)	8.7 (3.5, 13.5)	8.9 (2.9, 13.2)	0.034	0.44[b]	0.45[b]	77	5
Cholesterol (mg/d)	368.9 (150.3, 672.2)	338.0 (109.2, 689.1)	0.143	0.50[b]	0.44[b]	83	6
Calcium (mg/d)	517.4 (290.2, 842.8)	567.7 (302.7, 922.2)	<0.001	0.59[b]	0.49[b]	87	5
Magnesium (mg/d)	184.0 (91.2, 278.6)	212.5 (130.8, 319.7)	<0.001	0.40[b]	0.39[bc]	84	7
Fibre (g/d)	6.0 (2.9,10.2)	8.5 (3.2, 15.9)	<0.001	0.59[b]	0.31[b]	88	3
Sodium (mg/d)	3,641.5 (1,841.4, 5,972.8)	4,080.0 (1,494.9, 6,561.4)	0.001	0.50[b]	0.45[b]	80	3
Iron (mg/d)	21.8 (11.3, 35.5)	22.6 (10.9, 33.8)	0.016	0.66[b]	0.28[b]	87	1
Vitamin C (mg/d)	110.8 (41.8, 199.6)	144.5 (29.1, 370.0)	0.001	0.40[b]	0.38[b]	77	4
Cereal products	615.9 (312.5, 883.9)	589.2 (221.9, 862.0)	0.170	0.53[b]	0.50[b]	77	5
Meats	130.2 (33.7, 305.6)	121.1 (15.0, 296.2)	0.098	0.48[b]	0.41[b]	83	6
Fish and other seafood	84.9 (23.4, 154.3)	87.8 (22.2, 163.1)	0.644	0.57[b]	0.58[b]	85	4
Eggs	30.8 (8.6, 72.4)	31.9 (2.8, 80.7)	0.176	0.51[b]	0.41[b]	84	4
Vegetables	140.1 (50.8, 283.2)	172.0 (34.8, 381.9)	0.021	0.47[b]	0.44[b]	83	4
Legume	13.5 (0.0, 54.8)	14.0 (0.0, 44.1)	0.157	0.74[b]	0.70[b]	88	1
Bread spread	2.9 (0.0, 6.5)	10.5 (0.0, 21.5)	<0.001	0.23[b]	0.18[b]	63	9
Fruits	200.8 (48.3, 419.5)	326.8 (34.2, 765.4)	<0.001	0.20[b]	0.19[b]	72	7
Confectionaries	82.4 (18.3, 257.1)	80.5 (11.8, 257.1)	0.694	0.52[b]	0.55[b]	84	5
Fast food	21.1 (0.0, 42.9)	34.8 (0.0, 135.4)	0.004	0.65[b]	0.55[b]	88	1
Non-sugary drinks	642.5 (250.0, 2,000.0)	966.3 (0.0, 2,975.0)	<0.001	0.67[b]	0.66[b]	96	0
Sugary drinks	367.6 (43.9, 928.7)	337.4 (42.0, 849.2)	1.000	0.62[b]	0.61[b]	85	5
Alcohol drinks	25.1 (0.0, 35.0)	1.5 (0.0, 7.8)	0.172	0.64[b]	0.88[b]	-	-
Condiments	17.9 (2.2, 36.0)	36.8 (7.4, 72.7)	<0.001	0.22	0.22[b]	69	7
Dairy products	8.6 (0.0, 49.5)	8.4 (0.0, 50.0)	0.843	0.84[b]	0.83[b]	93	1

[a]Differences between FFQ1 and FFQ2 using Wilcoxon signed rank test; FFQ1: Food Frequency Questionnaire 1; FFQ2: Food Frequency Questionnaire 2
[b]$p<.05$
[c]Pearson correlation due to normal distribution data

the FFQ1 and FFQ2. Relatively moderate correlation on macronutrients between FFQ1 and FFQ2 from 0.45 (carbohydrate) to 0.64 (protein). Majority of nutrients and food groups do not exhibit significant difference between FFQs. Correlation for micronutrients ranged from 0.40 (magnesium) to 0.66 (iron) whereas food groups ranged from 0.20 (fruits) to 0.84 (dairy products). The lowest correlation coefficient for food groups was 0.20 compared to nutrients possibly due to more variances in food groups as opposed to the nutrient intake. In another study, fruits intake had over-estimation may due to complicacy in translating the stated dietary values into the real dietary intakes (Loy *et al.* 2011). However, true validity of FFQ1 is similar to that of FFQ2 against FR due to its highly correlated between FFQs. Classification into the same and adjacent quartile more than 63% for nutrients and food groups also may come from the short duration of administration between FFQs. Thus, the FFQ is found to be reproducible.

Validity

The FFQ had significantly higher estimates of most nutrients' and food groups' intake than the FR (Table 3). A systematic review showed that the overestimated nutrient and food groups from FFQ with respect to the reference method has been reported in several studies which could also be observed in the current study (Sierra-Ruelas *et al.* 2021). The overestimation FFQ intake can be clarified by the extensive list of food items and predetermined portion sizes compared to actual intake in FR (Loy *et al*. 2011). Some of the food items consumed in the FFQ was not consumed in FR which may lead to overestimation (Tollosa *et al.* 2017). However, only sugary drinks mean intake from FFQ lower from FR (Table 3), in line with previous study showed lower mean intake of soft drinks (with sugar) from FFQ than 4 days FR among healthy population in Jena, Germany (Steinemann *et al.* 2017).

In a systematic review on semi-quantitative FFQ validation among adults reported the most frequently mentioned elements by the studies were energy, macronutrients, saturated fatty acid, polyunsaturated fatty acid, cholesterol, calcium, fibre, vitamin C and iron (Sierra-Ruelas *et al.* 2021). This present study reported a weak to moderate correlation coefficient for nutrients and food groups derived from FFQ and FR significantly ranged from 0.23 to 0.64. Spearman coefficients were mostly applied to evaluate the relation strength linking FFQ and FR as it is less sensitive to extreme values compared to Pearson coefficients. Absolute magnitude in interpreting a correlation coefficient as follows: 0.00−0.10 as negligible; 0.10−0.39 as weak; 0.40−0.69 as moderate; 0.70−0.89 as strong and 0.90−1.00 as very strong correlation.

Energy adjustment reduced and did not improve the correlation for almost all nutrients in this study. A consistent outcome was also revealed in other FFQ validation studies (Kaur *et al*. 2016; Loy *et al.* 2011). The present findings are comparable to other studies conducted among adults or older adults in systematic review. Previous FFQ validation studies reported the lowest correlation coefficient of 0.28 for energy, 0.30 for protein, 0.17 for fat and 0.24 for carbohydrate (Sierra-Ruelas *et al.* 2021). The weak correlations for food groups of cereals products (0.33), meats (0.29), fish and seafoods (0.25), fruits (0.26), confectionaries (0.35), sugary drinks (0.28) and condiments (0.34) may be explained by the complicacy in translating the amount consumed into the real intakes and higher variations in food groups (Loy *et al.* 2011). Eggs, legume and bread spread were not significantly correlated between FFQ and FR may be due to periodically consumed group of food instead of frequently consumed and these food groups may not have been consumed during three days FR compared to the FFQ. Correlation should not be used alone to assess validity as it provides only the association degree between two methods. Therefore, the Bland–Altman method often practiced together to measure the agreement linking two methods rather than correlation only (Lee & Park 2016).

Energy had the largest percentage of respondents classified into the same and adjacent quartiles (75%) while calcium had the lowest (62%). Only four nutrients had more than 10% gross classification, indicating that misclassification was low (monounsaturated fatty acids 11%, polyunsaturated fatty acids 11%, fibre 11% and sodium 12%). For food groups, correctly classification had an average 74% respondents (58% to 92%) while mean of gross classification was 7%. Cross-classification provide a more accurate view of the FFQ's performance than the correlation coefficients (Loy *et al.* 2011).

Table 3. Validation of nutrient and food group between FFQ1 and FR (n=100)

Energy, nutrients, and food groups	FFQ1 (n=100)	FFQ2 (n=100)	p[a]	Spearman correlation		Cross-classification into quartiles (%)	
	Mean (P_5, P_{95})	Mean (P_5, P_{95})		Unadjusted	Energy adjusted	Correctly classified	Grossly misclassified
Energy (kcal)	2,352 (1,338, 3,393)	1,678 (1,110, 2,300)	<0.001	0.25[b]	-	75	0
Protein (g)	97.2 (54.8, 145.7)	71.8 (41.1, 92.4)	<0.001	0.27[b]	0.44[b]	41	9
Carbohydrate (g)	312.6 (191.5, 460.6)	221.7 (153.3, 299.1)	<0.001	0.29[b]	0.11	74	9
Fat (g)	75.7 (38.8, 119.0)	55.6 (28.3, 89.6)	<0.001	0.17	0.13[c]	63	7
Saturated fat (g)	12.8 (6.0, 22.1)	9.3 (3.1, 18.2)	<0.001	0.25[b]	0.26[b]	66	6
Monounsaturated fatty acids (g)	12.4 (6.2, 21.4)	9.0 (3.5, 16.6)	<0.001	0.14	0.26[b]	66	11
Polyunsaturated fatty acids (g)	8.7 (3.5, 13.5)	7.2 (2.2, 13.1)	0.001	0.06	0.16	66	11
Cholesterol (mg/d)	368.9 (150.3, 672.2)	292.8 (82.8, 552.3)	0.001	0.23[b]	0.26[b]	67	8
Calcium (mg/d)	517.4 (290.2, 842.8)	463.7 (262.1, 784.4)	0.017	0.10	0.06	62	7
Magnesium (mg/d)	184.0 (91.2, 278.6)	126.3 (63.2, 190.2)	<0.001	0.25[b]	0.26[bc]	68	9
Fibre (g/d)	6.0 (2.9,10.2)	3.8 (1.4, 7.3)	<0.001	0.12	0.23	64	11
Sodium (mg/d)	3,641.5 (1,841.4, 5,972.8)	3,398.8 (1,818.1, 5,349.8)	0.212	0.12	0.28[b]	73	12
Iron (mg/d)	21.8 (11.3, 35.5)	17.9 (8.1, 29.8)	<0.001	0.16	0.16	64	10
Vitamin C (mg/d)	110.8 (41.8, 199.6)	63.9 (11.2, 114.4)	<0.001	0.08	0.13	66	9
Cereal products	615.9 (312.5, 883.9)	520.7 (284.1, 772.9)	<0.001	0.33[b]	0.30[bc]	73	5
Meats	130.2 (33.7, 305.6)	119.2 (0.0, 268.6)	0.388	0.29[b]	0.17	72	8
Fish and other seafood	84.9 (23.4, 154.3)	69.9 (9.0, 165.2)	0.004	0.25[b]	0.20[b]	73	6
Eggs	30.8 (8.6, 72.4)	25.8 (0.0, 64.8)	0.149	-0.04	-0.03	58	13
Vegetables	140.1 (50.8, 283.2)	117.6 (5.6, 237.9)	0.007	0.55[b]	0.51[b]	89	4
Legume	13.5 (0.0, 54.8)	8.4 (0.0, 50.8)	0.003	0.07	0.15	68	7
Bread spread	2.9 (0.0, 6.5)	1.8 (0.0, 8.2)	<0.001	0.12	0.02	74	7
Fruits	200.8 (48.3, 419.5)	109.4 (0.0, 314.9	<0.001	0.26[b]	0.24[b]	70	11
Confectionaries	82.4 (18.3, 257.1)	64.6 (0.0, 203.2)	0.009	0.35[b]	0.40[b]	71	7
Fast food	21.1 (0.0, 42.9)	11.0 (0.0, 79.9)	<0.001	0.41[b]	0.21[b]	74	0
Non-sugary drinks	642.5 (250.0, 2000.0)	491.7 (166.7, 1166.7)	0.006	0.64[b]	0.59[b]	92	5
Sugary drinks	367.6 (43.9, 928.7)	388.3 (80.9, 764.6)	0.030	0.28[b]	0.23[b]	74	8
Alcohol drinks	25.1 (0.0, 35.0)	0.0 (0.0, 0.0)	0.018	-	-	-	-
Condiments	17.9 (2.2, 36.0)	15.9 (0.0, 42.0)	0.124	0.34[b]	0.35[b]	72	7
Dairy products	8.6 (0.0, 49.5)	29.9 (0.0, 223.4)	0.413	0.24[b]	0.07	47	18

[a]Differences between FFQ1 and FFQ2 using Wilcoxon signed rank test; FFQ1: Food Frequency Questionnaire 1; FFQ2: Food Frequency Questionnaire 2
[b]$p<0.05$
[c]Pearson correlation due to normal distribution data

The FFQ performance was account as valid if respondents were correctly classified into ≥50% and ≤10% grossly misclassified of the tertiles (Kaur *et al.* 2016). Similar result by other validation study among multi-ethnic population in the Malaysian Cohort Project varied showed cross-classification into adjacent quartile varied from 62.5 to 81.3% of the respondents (Shahar *et al.* 2021). Gross classification less than 10% of the tertiles are acceptable, in line with the present findings except for 4 nutrients. These findings suggest that the FFQ can rank respondents based on their dietary intake, which is significant for future colorectal cohort studies directing to determine the diet-disease association.

The Blant Altman plot (Figure 1) advanced to distinguish between FFQ and FR where the scattered plots were predominantly distributed for macronutrients within 95% level of agreement. As the scatter plot was primarily between the dotted lines (mean±2SD), the limit of agreement was considered to be fair. The Bland-Altman plot was

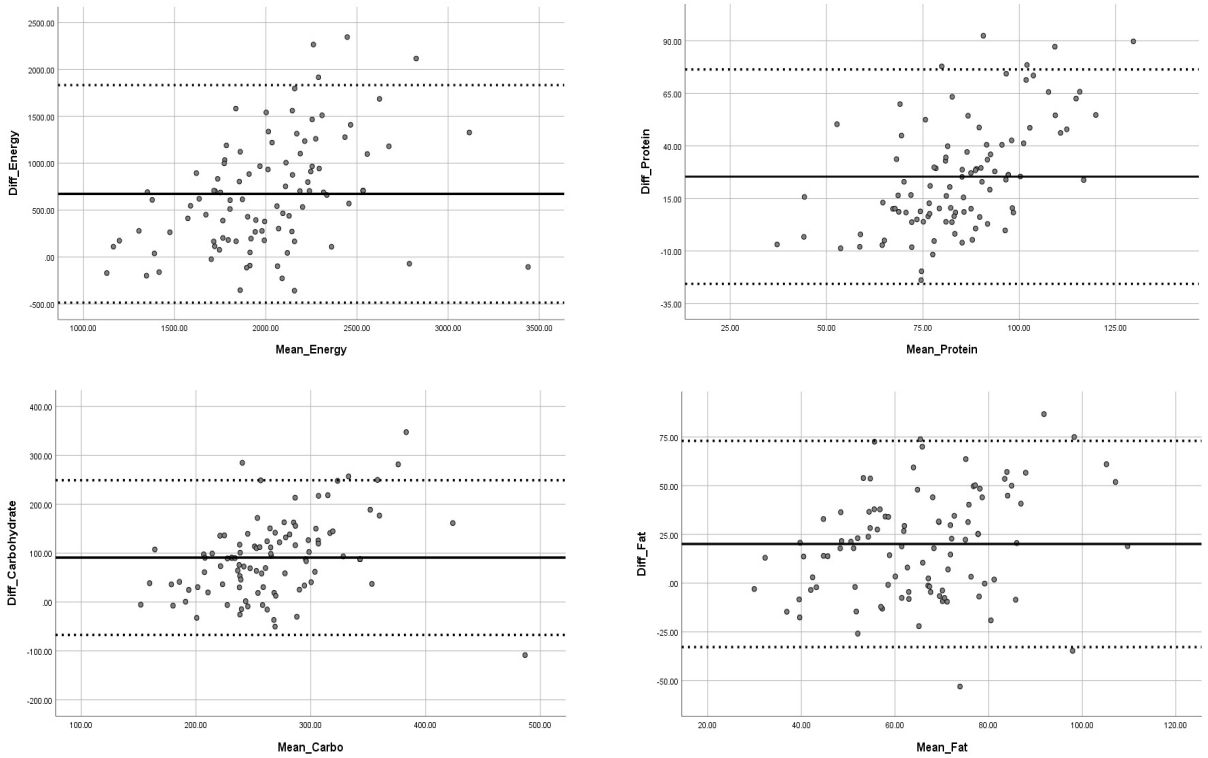

Figure 1. Bland altman plots for energy and macronutrients between food frequency questionnaire and food record (n=100)

used to interpret the agreement between FFQ and FR graphically at the group level, while correlation coefficient and cross-classification assess validity at the individual level (Harmouche-Karaki *et al.* 2020). Inconsistency was observed across the intake level of macronutrients whereby the mean differences increased as the level of intake further increased, representing the agreement between two methods was better at lower rather than higher, average intake values. Findings reporting no systematic errors for most nutrients in Bland-Altman plot are consistent with the present study (Knudsen *et al.* 2016; Yuan *et al.* 2017; Denova-Gutiérrez *et al.* 2016; Bijani *et al.* 2018; Beck *et al.* 2020).

Strengths of the present study are as follow: the use of 3 non-consecutive days of FR and multiple statistical analysis to determine validity and reproducibility of the FFQ. In addition, the sample size of the present study corresponds to the recommendation (n= 50−100) of FFQ validation studies (Cade *et al.* 2004) and the respondents were based on interview administered FFQ. Diversifying respondents during sampling method from a wider geographical area of urban and suburban area to represent Malaysia also part of the study strengths. However, there are some limitations could be addressed. First, the 142-food items FFQ might give burden to the respondent but no respondent withdrawal from implausible intake indicates satisfactory compliance of the respondents. Secondly, 3-day FR has limitation for estimating usual intake of foods not eaten daily or periodically consumed. Replicating the FR to 5 days may help in generating usual consumption. Third, the present study could be incorporating nutrient biomarker to strengthen the nutrient assessment of FFQ validation.

CONCLUSION

In conclusion, this FFQ is a valid and reproducible instrument to determine habitual intake among CRC patients in Malaysia. This FFQ is capable well to assess the energy, nutrients and food groups related to cancer and rank individuals by relative intake level. Thus, this FFQ is recommended as a valid instrument in a CRC prospective study for dietary data collection.

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DECLARATION OF INTERESTS

The authors have no conflicts of interest to disclose.

REFERENCES

Beck KL, Houston ZL, Mcnaughton SA, Kruger R. 2020. Development and evaluation of a food frequency questionnaire to assess nutrient intakes of adult women in New Zealand. Nutr Diet 77(2):253−259. https://doi.org/10.1111/1747-0080.12472

Bijani A, Esmaili H, Ghadimi R, Babazadeh A, Rezaei R, Cumming RG, Hosseini SR. 2018. Development and validation of a Semi-quantitative food frequency questionnaire among older people in north of Iran. Caspian J Intern Med 9(1):78. https://doi.org/ 10.22088/cjim.9.1.78

Cade JE, Burley VJ, Warm DL, Thompson RL, Margetts BM. 2004. Food-frequency questionnaires: A review of their design, validation and utilisation. Nutr Res Rev 17(1):5−22. https://doi.org/10.1079/NRR200370

Denova-Gutiérrez E, Ramírez-Silva I, Rodríguez-Ramírez S, Jiménez-Aguilar A, Shamah-Levy T, Rivera-Dommarco JA. 2016. Validity of a food frequency questionnaire to assess food intake in Mexican adolescent and adult population. Salud Publica Mex 58:617−628. https://doi.org/10.21149/spm.v58i6.7862

El Kinany K, Garcia-Larsen V, Khalis M, Deoula MMS, Benslimane A, Ibrahim A, Benjelloun MC, El Rhazi K. 2018. Adaptation and validation of a food frequency questionnaire (FFQ) to assess dietary intake in Moroccan adults. Nutr J 17(1):1−12. https://doi.org/10.1186/s12937-018-0368-4

Harmouche-Karaki M, Mahfouz M, Obeyd J, Salameh P, Mahfouz Y, Helou K. 2020. Development and validation of a quantitative food frequency questionnaire to assess dietary intake among Lebanese adults. Nutr J 19(1):1−19. https://doi.org/10.1186/s12937-020-00581-5

Henriksen HB, Carlsen MH, Paur I, Berntsen S, Bøhn SK, Skjetne AJ, Kværner AS, Henriksen C, Andersen LF, Smeland S, *et al.* 2018. Relative validity of a short food frequency questionnaire assessing adherence to the Norwegian dietary guidelines among colorectal cancer patients. Food Nutr Res 62. https://doi.org/10.29219/fnr.v62.1306

[IPH] Institute for Public Health. 2011. Album Makanan Malaysia. Malaysia (MY): Institue for Public Health, Ministry of Health Malaysia.

Ibrahim NRW, Chan HK, Soelar SA, Azmi AN, Said RM, Hassan MRA. 2020. Incidence, clinico-demographic profiles and survival rates of colorectal cancer in Northern Malaysia: Comparing patients above and below 50 years of age. Asian Pac J Cancer Prev 21(4):1057−1061. https://doi.org/10.31557/APJCP.2020.21.4.1057

International Agency for Research on Cancer. 2020. Lyon: International association of cancer registries. https://gco.iarc.fr/today/home [Accessed June 25 2021].

Kaur S, Mohamed HJBJ, Jalil RA, Yusof BNM, Yim HS. 2016. Validation and reproducibility of a culturally specific food frequency questionnaire (FFQ) for Malaysian Punjabis. Malays J Nutr 22(2):245−255.

Knudsen VK, Hatch EE, Cueto H, Tucker KL, Wise L, Christensen T, Mikkelsen EM. 2016. Relative validity of a semi-quantitative, web-based FFQ used in the 'Snart Forældre'cohort-a Danish study of diet and fertility. Public Health Nutr 19(6):1027−1034. https://doi.org/10.1017/S1368980015002189

Koole JL, Bours MJ, Breedveld-Peters JJ, van Roekel EH, van Dongen MC, Eussen SJ, van Zutphen M, van Duijnhoven FJ, Boshuizen HC, Weijenberg MP. 2020. Evaluating the validity of a food frequency questionnaire in comparison with a 7-day dietary record for measuring dietary intake in a population of survivors of colorectal

cancer. J Acad Nutr Diet 120(2):245−257. https://doi.org/10.1016/j.jand.2019.09.008

Lee Y, Park K. 2016. Reproducibility and validity of a semi-quantitative FFQ for trace elements. Br J Nutr 116(5):864−873. https://doi.org/10.1017/S0007114516002622

Liu L, Wang PP, Roebothan B, Ryan A, Tucker CS, Colbourne J, Baker N, Cotterchio M, Yi Y, Sun G. 2013. Assessing the validity of a self-administered food-frequency questionnaire (FFQ) in the adult population of Newfoundland and Labrador, Canada. Nutr J 12(1):1−9. https://doi.org/10.1186/1475-2891-12-49

Loy S, Marhazlina M, Azwany Y, Jan JM. 2011. Development, validity and reproducibility of a food frequency questionnaire in pregnancy for the Universiti Sains Malaysia birth cohort study. Malays J Nutr 17(1):1−18.

Luftimas DE, Lucita A, Wiramihardja S, Rusmil VK. 2021. Validation of iron-food frequency questionnaire for assessing iron intake in women of reproductive age: A cross-sectional study on female undergraduate students in Indonesia. J Gizi Pangan 16(2):63−70. https://doi.org/10.25182/jgp.2021.16.2.63-70

Mcnabb S, Harrison TA, Albanes D, Berndt SI, Brenner H, Caan BJ, Campbell PT, Cao Y, Chang-Claude J, Chan A, *et al*. 2020. Meta-analysis of 16 studies of the association of alcohol with colorectal cancer. Int J Cancer Res 146(3):861−873. https://doi.org/10.1002/ijc.32377

Morze J, Danielewicz A, Przybyłowicz K, Zeng H, Hoffmann G, Schwingshackl L. 2021. An updated systematic review and meta-analysis on adherence to mediterranean diet and risk of cancer. Eur J Nutr 60(3):1561−1586. https://doi.org/10.1007/s00394-020-02346-6

National Cancer Institute 2019. Malaysian National Cancer Registry Report (2012−2016). 2019: Malaysia (MY): Ministry of Health Malaysia.

Ocké MC, De Vries JHM, Hulshof PJM. 2020. Assessment of Dietary Intake by Self-reports and Biological Markers. In Present Knowledge in Nutrition 11 Edition. Massachusetts (USA): Academic Press.

Rawla P, Sunkara T, Barsouk A. 2019. Epidemiology of colorectal cancer: Incidence, mortality, survival, and risk factors. Prz Gastroenterol 14(2):89−103. https://doi.org/10.5114/pg.2018.81072

Shahar S, Shahril MR, Abdullah N, Borhanuddin B, Kamaruddin MA, Yusuf NAM, Dauni A, Rosli H, Zainuddin NS, Jamal R. 2021. Development and relative validity of a semiquantitative food frequency questionnaire to Estimate dietary intake among a multi-ethnic population in the Malaysian cohort project. Nutrients 13(4):1163. https://doi.org/10.3390/nu13041163

Sierra-Ruelas É, Bernal-Orozco MF, Macedo-Ojeda G, Márquez-Sandoval YF, Altamirano-Martínez MB, Vizmanos B. 2021. Validation of semiquantitative FFQ administered to adults: A systematic review. Public Health Nutr 24(11):3399−3418. https://doi.org/10.1017/S1368980020001834

Steinemann N, Grize L, Ziesemer K, Kauf P, Probst-Hensch N, Brombach C. 2017. Relative validation of a food frequency questionnaire to estimate food intake in an adult population. Food Nutr Res 61(1):1305193. https://doi.org/10.1080/16546628.2017.1305193

Tollosa DN, Van Camp J, Huybrechts I, Huybregts L, Van Loco J, De Smet S, Sterck E, Rabâi C, Van Hecke T, Vanhaecke L. 2017. Validity and reproducibility of a food frequency questionnaire for dietary factors related to colorectal cancer. Nutrients 9(11):1257. https://doi.org/10.3390/nu9111257

van Dongen MC, Wijckmans-Duysens NE, den Biggelaar LJ, Ocké MC, Meijboom S, Brants HA, de Vries JH, Feskens EJ, Bueno-de-Mesquita HB, Geelen A, *et al.* 2019. The maastricht FFQ: Development and validation of a comprehensive food frequency questionnaire for the maastricht study. Nutr 62:39−46. https://doi.org/10.1016/j.nut.2018.10.015

Willett W. 2012. Nutritional Epidemiology. New York (USA): Oxford University Press.

World Cancer Research Fund. 2018. Diet, Nutrition, Physical Activity and Colorectal Cancer. Revised 2018. London (UK): American Institute for Cancer Research.

Yuan C, Spiegelman D, Rimm EB, Rosner BA, Stampfer MJ, Barnett JB, Chavarro JE, Subar AF, Sampson LK, Willett WC. 2017. Validity of a dietary questionnaire assessed by comparison with multiple weighed dietary records or 24-hour recalls. Am J Epidemiol 185(7):570−584. https://doi.org/10.1093/aje/kww104

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Lipid Profile Improving Effect of Tenggulun Leaf (*Protium javanicum*) Tea Powder in Rats Fed with High-Fat Diet

Ni Luh Ari Yusasrini*, Luh Putu Trisna Darmayanti
[1]Department of Food Technology, Faculty of Agricultural Technology, Udayana University, Badung 80361, Indonesia

ABSTRACT

The objective of this research was to determine the effect of tenggulun leaf tea powder in improving lipid profile in rats fed with high fat diet. We developed a tenggulun leaf tea powder and did a biochemical analysis. The in vivo study was conducted using 18 male Wistar rats grouped into three different diets, Control-Standard Feed (CSF) group, Hypercholesterol-Standard Feed (HSF) group and Hypercholesterolemia-Tenggulun Feed (HTF) group. The treatment was given for 30 days in all groups. Biochemical analysis showed that tenggulun leaf tea powder contains various cholesterol lowering substance such as dietary fibre (42.73%), phenolic content (9.42 mg GAE/g), tannins (10.80 mg TAE/g), flavonoids content (1.81 mg QE/g), and it also showed antioxidant activity (IC_{50} value of 67.20 ppm). In vivo analysis after treatment showed that there was no significant difference in total cholesterol levels between the HSF and HTF groups. However, in the HTF group there was a decrease in total cholesterol levels from the initial level by 16.48%. In addition, the HTF group had significantly higher HDL and lower LDL level compared to the HSF. The administration of tenggulun leaf tea powder for 30 days showed a significant effect on Low-Density Lipoprotein (LDL) and High-Density Lipoprotein (HDL) cholesterol levels, but not on serum Triglyceride (TG) levels. Therefore, the tenggulun leaf tea powder showed a significant effect in improving lipid profile in rats fed with high fat diet.

Keywords: cholesterol, *protium javanicum,* tea, tenggulun

INTRODUCTION

Herbal tea is products made from the leaves, stems, flowers, or seeds of one or a mixture of several herbal plants (Ravikumar 2014). Recently, herbal teas have received a lot of attention because of their beneficial physiological effects on health. One of the plants that have the potential to be developed into herbal teas is tenggulun (*Protium javanicum*). Tenggulun is a tropical plant with leaf morphology that is not too wide, tapered, and pink on very young leaves and dark green on old leaves. In Bali, young tenggulun leaves are often consumed as vegetables and used for traditional medicine.

Tenggulun leaves contain various bioactive compounds such as phenolics, flavonoids, and tannins (Simamora *et al.* 2021), α and β amyrin, and β sitosterol (Puspawati *et al.* 2019). Santos *et al.* (2012) reported that protium genera such as *Protium heptaphyllum* has antihyperglycemic and hypolipidemic effects. The content of triterpene compounds (α and β amyrin mixture) in these genera could be a leading compound for drug development effective in diabetes and atherosclerosis. The content of other bioactive compounds such as dietary fibre, flavonoids and tannins can prevent and treat degenerative diseases. Maheshwari (2020) also reported that various mixtures of phytochemical (plant sterols, flavonoid, lignan) extracts that can help in reducing blood cholesterol levels.

Tenggulun leaf tea powder is an herbal product made from young tenggulun leaves. The leaves are steamed, dried, grinded, and sifted to obtain a fine powder. It can be consumed by dissolving it in water without being filtered. Rohiqi *et al.* (2021) reported that the best tenggulun leaf tea powder comes from young leaves. Other researchers reported that steaming method was the best processing for producing tenggulun leaf tea powder (Yusasrini & Permana 2021).

Various studies have been carried out to explore the potential of herbal tea products for the treatment or prevention of hypercholesterolemia

*Corresponding Author: tel: +6287860462552, email: ariyusasrini@unud.ac.id
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such as *Curcuma zedoaria* Roscoe tea (Tariq *et al.* 2016) and green grass jelly leaf tea (Rizki *et al.* 2015). Kuchta *et al.* (2021) reported that administration of *Cistus incanus* tea containing phenols and flavonoids could improve lipid profiles in healthy volunteers. Giving a combination of white tea and Moringa leaves has also been reported to reduce blood triglycerides in vivo (Martini *et al.* 2019). *Cang salak* tea (Karta *et al.* 2021) and chamomile tea (Kaseb *et al.* 2018) have also been reported to improve lipid profiles in hypercholesterolemic conditon.

Despite the vast body of evidence for other herbal tea products for improving cholesterol profile, studies on the physiological effects of tenggulun leaf tea powder in vivo have been absent. It is important to explore more on the potential of this local plants for the prevention or treatment of hypercholesterolemia. Therefore, this research aimed to determine the effect of tenggulun leaf tea powder in improving lipid profile in rats fed with a high-fat diet.

METHODS

Design, location, and time

This research was carried out at the Food Processing Laboratory, and at the Biochemistry and Nutrition Laboratory, Faculty of Agricultural Technology, Udayana University from March to September 2021. This is an experimental study with a post-test control group design. The completely randomized design used 3 treatment groups and 6 replications so that 18 experimental units were obtained. This research has been approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Udayana University with a Certificate of Approval of Animal Ethics Number: B/146 /UN14.2.9/ PT.01.04/2021.

Material and tools

Young tenggulun leaves with characteristic light green color, limp and spotless, that grows wild in the Bukit Jimbaran area were collected. The standard feed ingredients according to AIN 93 consists of 62.069% corn starch, 5% Carboxymethyl Cellulose (CMC), 4% soybean oil, 10% sucrose, 14% casein, 1% vitamin mix, 3.5% mineral mix, 0.18% L-cystine, and 0.25% choline bitartrate. The reagents for analysis were cholesterol FS (Diasys), triglycerides FS (Diasys), HDL Precipitant (Diasys), 70% ethanol, DPPH (2,2-diphenyl-1-picrylhydrazyl), $NaNO_2$, $AlCl_3$, NaOH, H_2SO_4, boric acid, HgO, Na_2SO_4, HCl, and hexane.

The equipment used for the research included a vortex, oven, small centrifuge (Hettich EBA III), Eppendorf tubes, blender, a set of individual mouse cages, muffle furnace (Heraeus Instrument), oven, analytical balance (Sartorius), injection syringe, micro hematocrite tube (Becton Dickinson & Company), micro-pipette and glassware.

Procedure

Material preparation. Tenggulun leaf tea powder was obtained using the modified steaming method according to Topuz *et al.* (2014). A batch of 100 g of young tenggulun leaves were collected and then steamed at 100°C for 90 seconds. After the tenggulun leaves had been cooled for 5 min, it was dried in an oven at 50°C for 4 h. The dried tenggulun leaves was then ground and sieved through a 100-mesh sieve.

Feed formulation. Standard feed was made by mixing ingredients according to the standard AIN 1993. The standard feed composition per kg consisted of 620.69 g corn starch, 140 g casein, 100 g sucrose, 40 g soybean oil, 50 g Carboxymethyl Cellulose (CMC), 35 g mineral mixture, 10 g vitamin mix, 1.8 g L-cystine, and 2.5 g of choline bitartrate. High-fat feed was prepared by adding 2% cholesterol and 10% beef fat per kg of standard feed. Tenggulun leaf tea powder feed was made by substituting 10% tenggulun leaf tea powder into a standard feed mixture with isocaloric considerations. To obtain the isocaloric value, the amount of corn starch, casein, soybean oil, CMC, and mineral mix in the tenggulun leaf tea powder feed was determined based on the results of the proximate analysis of the tenggulun leaf tea powder. Each feed ingredient was mixed homogeneously, put into a printing machine, and dried in an oven at a temperature of 50^0C for 4 h.

Preparation of experimental animals. Eighteen (18) male Wistar rats with initial weight of 100±5 g were placed in individual cages and acclimatized for one week with standard feed and drinking water ad libitum. Furthermore, the rats were divided into three groups (each group consisted of 6 rats) namely: 1). Control-Standard Feed (CSF), 2). Hypercholesterol-Standard Feed

(HSF), and 3). Hypercholesterol-Tenggulun Feed (HTF). The CSF group was given standard feed from the beginning to the end of the bioassay. The HSF group was fed a high-fat diet for two weeks, followed by a standard diet for 30 days. The HTF group was fed a high-fat diet for two weeks, then fed tenggulun leaf tea powder feed for 30 days. Bioassays were carried out for 30 days. At the end of the bioassay, blood was collected for analysis.

Blood collection. Blood was collected after two weeks of high-fat feeding for initial cholesterol level determination, and at the end of the bioassay for lipid profile analysis. Before blood collection, rats were fasted overnight. Blood was taken from the orbital sinus of the eye, collected in Eppendorf tubes, and centrifuged at 4,000 rpm for 10 min. Blood serum was accommodated in microtubes and analysed for lipid profiles.

Proximate analysis. Proximate analysis was carried out according to AOAC (2005). The moisture and ash content analysis were carried out according to the gravimetric method, the protein content analysis was carried out using the semi-micro Kjeldahl method and the fat analysis was carried out according to the Soxhlet method. Carbohydrate content was calculated by difference. Analysis of dietary fibre was carried out using the multienzyme method according to AOAC (2005).

Total phenolic content analysis. Analysis of total phenolic content was carried out using the spectrophotometric method according to Sakanaka *et al.* (2005) with slight modifications. A total of 0.1 g of the sample was put into a tube and diluted with 85% ethanol to a volume of 5 ml. The solution was vortexed until homogeneous and centrifuged at 2,500 rpm for 15 min. A total of 50 µl of filtrate was transferred into a test tube and added with 350 µL of distilled water. The mixture was added with 400 ml of folin ciocalteu reagent. Incubated for 6 min, and added with 4.2 ml of 5% Na_2CO_3. The solution was vortexed and incubated again for 90 min. The absorbance was read at a wavelength of 760 nm.

Total flavonoid analysis. Total flavonoid analysis was carried out using the spectrophotometric method according to da Silva *et al.* (2015) with slight modifications. A total of 5 g sample was dissolved in 100 ml of ethanol. A total of 0.1 g of dissolved sample was weighed and added with 50% ethanol until the volume was 5 ml. The solution was vortexed until homogeneous and then centrifuged at 2,500 rpm for 15 min. A total of 50 µl of filtrate was transferred into a test tube and added with 450 µl of ethanol and 1 ml of $AlCl_3$. The solution was incubated for 30 min. The absorbance was read at a wavelength of 415 nm.

Tannin analysis. Tannin analysis was carried out using the spectrophotometric method according to Nair *et al.* (2015) with slight modified. A total of 5 g of the sample that has been mashed was added with distilled water to a volume of 100 ml. The solution was shaken until homogeneous and filtered. A total of 1 ml of clear solution was added with 0.5 ml of follin denis solution, 1 ml of saturated $NaCO_3$ solution, and distilled water until it reached 10 ml in volume. The solution was vortexed until homogeneous and absorbance was read at a wavelength of 730 nm.

Antioxidant activity. Determination of antioxidant activity was carried out by the DPPH (1,1,2,2-Diphenyl Picryl Hydrazyl) method according to Molyneux (2004). Samples were made with certain concentrations. A total of 1 ml of each solution was put in a test tube and added with 1 ml of the 200 mm DPPH. The solution was incubated in the dark for 30 min and diluted with methanol to a volume of 5 ml. The absorbance of the solution was measured at a wavelength of 517 nm.

Total cholesterol analysis. Total cholesterol was measured according to the CHOD-PAP enzymatic photometric method following the procedure from Kit DiaSys®, Germany (Diagnostic System International). A total of 10 µl of serum was added to 1,000 µl of reagent, then incubated for 20 min at 25°C. The absorbance was read at a wavelength of 500 nm. Total cholesterol was calculated based on the formula:

$$Total\ cholesterol\left(mg/dl\right) = \frac{Sample\ absorbance}{Standard\ absorbance}\ X\ Standard\ concentration\left(\frac{mg}{dl}\right)$$

HDL cholesterol analysis. Analysis of HDL cholesterol using the CHOD-PAP enzymatic photometric method based on the procedure from Kit DiaSys®, Germany (Diagnostic System International). A total of 200 µl of serum was added with precipitate reagent, then incubated for 15 min at room temperature, centrifuged for 20 min at a speed of 2,500 rpm. A total of 100 µl of

supernatant was added to 1,000 µl of cholesterol reagent, then vortexed and incubated for 10 min at ambient temperature. The absorbance was read at a wavelength of 500 nm. HDL cholesterol was calculated based on the formula:

$$HDL-cholesterol\,(mg/dl) = \frac{Sample\ absorbance}{Standard\ absorbance}\ X\ Standard\ concentration\left(\frac{mg}{dl}\right)$$

LDL cholesterol analysis. LDL cholesterol was calculated using the formula compiled by Fridewald.

$$LDL-cholesterol = Total\ cholesterol - \left(HDL + \frac{1}{5}\ Triglycerides\right)$$

Total triglyceride analysis. Total triglyceride analysis was determined by the GPO-PAP method using the ReiGed Diagnostic Kit. A total of 10 µl of serum was added to 1,000 µl of reagent, then vortexed and incubated for 10 min at 25^{O}C. The absorbance was read at a wavelength of 505 nm. Total triglycerides were calculated based on the formula:

$$Total\ triglyceride\,(mg/dl) = \frac{Sample\ absorbance}{Standard\ absorbance}\ X\ Standard\ concentration\left(\frac{mg}{dl}\right)$$

Data analysis

The data on proximate analysis, dietary fiber, total phenol, total flavonoid, total tannin and antioxidant activity of tenggulun leaf tea powder was presented in descriptive analysis. Data on total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides between groups were analysed using ANOVA followed by Duncan post-hoc test by using the IBM SPSS 23.

RESULTS AND DISCUSSION

The chemical composition of tenggulun leaf tea powder

Tenggulun leaf tea powder has physical characteristics of yellowish green color, uniform particle size, and distinctive aroma of tenggulun leaves. The results of the proximate analysis of tenggulun leaf tea powder are presented in Table 1.

Tenggulun leaf tea powder has a low moisture content of 7.66% and complying to the national standard SNI 3836:2013 regarding packaged dry tea which requires a maximum moisture content of 8%. Moisture content is closely related to product quality. High water content affects the shelf life and, sensory properties such as the taste and aroma of steeping tea and causes microbiological contamination. The ash content of tenggulun leaf tea powder is 7.07%, which met the SNI requirements of maximum 8%.

Table 1. Chemical composition of tenggulun leaf powder

Chemical composition	Content (%)
Moisture content	7.66±0.07
Ash content	7.07±0.10
Protein content	12.2±0.04
Lipid content	0.53±0.01
Carbohydrate (by difference)	72.53±0.02

Tenggulun leaf tea powder contains 42.73% dietary fiber with the main components being insoluble dietary fiber 40.19% and soluble dietary fiber 2.53% (Table 2). The content of other bioactive compounds in tenggulun leaf tea powder is listed in Table 2.

Tenggulun leaf tea powder contains phenols, tannins, and flavonoids of 9.42, 10.8, and 1.815%, respectively. The level of phenol in this herbal tea product is lower than the polyphenol compound required by SNI 3945:2016, which is at least 15%. Bioactive components such as phenols, tannins, and flavonoids are related to the antioxidant activity of tenggulun leaf tea powder. The IC_{50} results of tenggulun leaf tea powder showed a value of 67.205 ppm, which indicates strong antioxidant activity. Antioxidant activity is classified as "strong" if the IC_{50} value is between 50–100 ppm (Molyneux 2004).

Phenolic compounds are the biggest group of phytochemicals. Their bioavailability is

Table 2. Dietary fiber, total phenol, total flavonoid, total tannin and antioxidant activity of tenggulun leaf powder

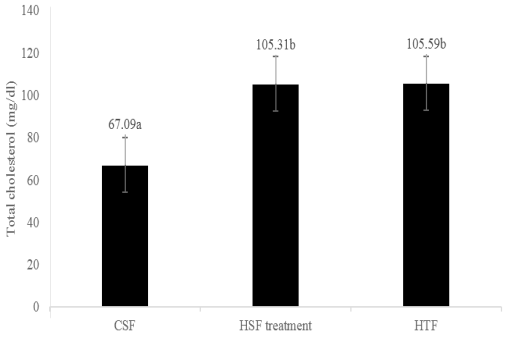
Bioactive compounds	Content
Total dietary fiber content (%)	42.73 ± 0.16
Soluble dietary fiber	2.53 ± 0.07
Insoluble dietary fiber	40.19 ± 0.09
Total phenolic content (mg GAE/g)	9.42 ± 0.08
Tannin content (mg TAE/g)	10.8 ± 0.08
Total flavonoids (mg QE/g)	1.815 ± 0.13
Antioxidant activity (IC_{50}) (ppm)	67.205 ± 1.71

decisive in exerting useful consequences in vivo and is encouraged through the molecular length and complexity of their chemical structure, consisting of conjugation with different phenols, polymerization, glycosylation, acylation, or hydroxylation (Sobhani *et al.* 2021). Flavonoids (consisting of flavonols, flavones, flavanones, flavanols, isoflavones, and anthocyanidins) are a collection of polyphenols that exert a couple of useful consequences. A current examination indicates that flavonols, particularly quercetin , alters the intestine microbiota and decreases the atherogenic lipids, including cholesterol and lysophosphatidic acids, with these types of consequences being related to the diminution of atherosclerotic lesions area (Nie *et al.* 2019). When compared with similar products, the total phenol content of tenggulun leaf tea powder is higher than African leaf tea powder (Putri *et al.* 2021) but lower than the total phenolic content of matoa leaf tea (Dewi *et al.* 2021).

Total cholesterol

Analysis of variance showed that rats fed with a high-fat diet had a significantly higher total cholesterol level compared to the control. The data is illustrated in Figure 1.

The HSF and HTF groups had higher baseline cholesterol levels (56.96–57.37%) than CSF group. The addition of 2% cholesterol and 10% beef fat into the standard feed was effective in increasing the cholesterol levels of rats. In mice *R. norvegicus* Wistar strain, normal blood cholesterol levels is 10–54 mg/dl. Beef fat contains higher saturated fatty acids than pork (Prabawati & Fajriati 2019). The dominant effect of these saturated fatty acids is an increase in total cholesterol level. The outcomes of this examination are in line with the ones reported by Dahlia *et al.* (2017) and Harsa (2014) that the administration of a high-fat diet was able to increase the total cholesterol levels of rats.



Bars with different letters show a significant difference at $p<0.05$ (n=6)

CSF: Control-Standard Feed; HSF: Hypercholesterolemia-Standard Feed; HTF: Hypercholesterolemia-Tenggulun Feed

Figure 1. Effect of high-fat diet on initial cholesterol levels

Analysis of variance showed that feeding of tenggulun leaf tea powder for 30 days could improve lipid profile in rats (Table 3). CSF group had the lowest total cholesterol level, while the HSF group had the highest, but there was no significant difference between the HSF and HTF groups. However, HTF group showed a decrease of 16.48% in total cholesterol compared to the initial cholesterol level.

Analysis of variance also showed that despite consuming the same high fat diet, the HTF group had significantly lower LDL level compared to the HSF group and it did not differ significantly with the CSF group that received normal diet. Therefore, it indicates that the feeding of tenggulun leaf tea powder had a significant effect on lowering LDL cholesterol levels in rats with high fat diet. In addition, the HTF group also had significantly higher HDL level compared to both the HSF and CSF groups. On the other hand, tenggulun leaf tea powder showed no significant effect on blood triglyceride levels in rats. Although statistically there has been no significant difference in serum triglyceride

Tabel 3. Effect of high-fat diet on the lipid profile of rats after 30 days of intervention

Treatments	Total cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	Triglyceride (mg/dl)
CSF	66.67±16.73[a]	6.46±5.26[a]	51.65±16.95[a]	43.26±10.35[a]
HSF	94.87±5.79[b]	28.76±16.54[b]	57.05±13.28[a]	45.26±24.73[a]
HTF	88.18±11.78[b]	3.45±3.14[a]	77.75±12.76[b]	34.86±7.80[a]

[a,b] in the same column show a significant difference at $p<0.05$ (n=6)

LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein; CSF: Control-Standard Feed; HSF: Hypercholesterolemia-Standard Feed; HTF: Hypercholesterolemia-Tenggulun Feed

between the three groups, the administration of tenggulun leaf tea powder tended to lower serum triglyceride level. The HTF group had 22.97% lower triglyceride levels than the HSF group and 19.41% lower than the CSF group.

The high content of dietary fibre and the presence of bioactive compounds such as phenols, tannins, and flavonoids in food can help improve blood lipid profile. Tenggulun leaf tea powder contains a high dietary fibre of 42.73% and is dominated by Insoluble Dietary Fibre (IDF). Dietary fibre has antihyperlipidemic properties through bulking effect, low ranges of energy, viscosity, fermentation, and binding capacity. Insoluble dietary fibre presents bulking effect, consequently growing stool mass, assuaging constipation, and enhancing regularity. Due to the extended bulk and water content, the nutrients including sugar and lipids, are diluted and their migration to the intestinal partitions slows down. The IDF also relates to a slower intestinal transit time that enables a lower absorption time of those sugars and lipids. Hence, it lowers the absorption of macronutrients, particularly carbohydrates and cholesterol, both through delaying gastric emptying or through shortening small intestinal transit time, similarly to a discounted glycaemic response, which can help the discount in insulin stimulation of hepatic cholesterol synthesis (Dai & Chau 2017; Nie *et al.* 2021). Dietary fiber also inhibits the absorption of cholesterol in the intestine, which causes increased excretion of bile acids through faeces so that more cholesterol is converted into bile acids to emulsify fats. These reasons explain lower overall LDL levels of cholesterol in the blood (Soliman 2019).

Tenggulun leaf tea powder also contains phenols, tannins, and flavonoids that function as antioxidants and are hypocholesterolaemic. Flavonoids have been reported to reduce oxidative stress and regulate blood lipid levels, exhibit anti-inflammatory and activities activity, and improve endothelial function (Siasos *et al.* 2013). As mentioned earlier, phenolic compounds in foods, especially in herbal teas are also reported to have hypocholesterolemic effect by inhibiting LDL oxidation, thereby minimizing the possibility of blood vessel damage caused by LDL oxidation (Amarowicz 2016). Dahlia *et al.* (2017) found that the administration of white tea extract can reduce LDL cholesterol in the blood. Likewise, as reported by Karta *et al.* (2021), *Cang salak* tea was able to normalize LDL cholesterol levels in hyperlipidemic rats. In addition, tannins, such as contained in the tenggulun tea also increases lipoprotein lipase activity resulting in decreasing total serum cholesterol (Kothari *et al.* 2011).

On the other hand, HDL has an antagonistic function to LDL. In the metabolic process, LDL primarily transports lipids from the intestine and liver to the peripheral tissues, while the HDL is primarily involved in reverse cholesterol transport (Bali & Utaal 2019). LDL is atherogenic because it causes calcification of the coronary vessels, in contrast to HDL that prevents the occurrence of calcification. HDL contains less than 25% cholesterol so it minimizes the danger of atherosclerosis. Our study showed that rats treated with high fat diet and tenggulun leaf tea powder had significantly higher HDL compared to rats receiving high fact diet without tenggulun tea. It is proposed that dietary fibre might indirectly able to increase blood HDL levels by lowering cholesterol absorption. In addition, protective function of flavonoid intake is related to the bioactivity of flavonoids as antioxidants and anti-inflammatory compounds. These characteristics can increase HDL or reverse cholesterol transport and provide protection against HDL dysfunction in the context of inflammatory disease states such as atherosclerosis or obesity (Millar *et al.* 2017).

CONCLUSION

The administration of tenggulun leaf tea powder at 10% of standard feed for 30 days to a group of rats fed with a high-fat diet improves the overall blood lipid profile. Rats fed with high fat diet containing tenggulun leaf tea powder had significantly lower LDL and higher HDL levels compared to groups that did not receive the herbal tea. However, it did not show significant difference in total cholesterol level and triglycerides. Hence, further research is needed to determine the appropriate dose of tenggulun leaf tea powder that are able to significantly reduce total cholesterol and triglycerides levels.

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DECLARATION OF INTERESTS

The authors declare that there is no conflict of interest with other person or institution.

REFERENCES

[AOAC] Association of Official Analytical Chemists. 2005. Official Methods of Analysis: Gathersburg (USA): Association of Official Analytical Chemists.

Amarowicz R. 2016. Natural phenolic compounds protect LDL against oxidation. Eur J Lipid Sci Technol 118(5):677–679. https://doi.org/10.1002/ejlt.201600077

Bali S, Utaal MS. 2019. Serum lipids and lipoproteins: A brief review of the composition, transport and physiological functions. Int J Sci Rep 5(10):309. https://doi.org/10.18203/issn.2454-2156.IntJSciRep20194253

da Silva LAL, Pezzini BR, Soares L. 2015. Spectrophotometric determination of the total flavonoid content in *Ocimum basilicum* L. (Lamiaceae) leaves. Pharmacogn Mag 11(41):96–101. https://doi.org/10.4103/0973-1296.149721

Dahlia D, Pangkahila WI, Aman IGM, Pangkahila JA, Suryadhi NT, Iswari IS. 2017. Pemberian ekstrak teh putih (*Camellia sinensis*) oral mencegah dislipidemia pada tikus putih (*Rattus novergicus*) galur wistar yang diberi diet tinggi lemak. J Anti-Aging Med 1(1):17–24

Dai FJ, Chau CF. 2017. Classification and regulatory perspectives of dietary fiber. J Food Drug Anal 25(1):37–42. https://doi.org/10.1016/j.jfda.2016.09.006

Dewi PL, Yusasrini NLA, Wisaniyasa NW. 2021. Pengaruh metode pengolahan terhadap aktivitas antioksidan dan karakteristik teh herbal daun matoa (*Pometia pinnata*). Jurnal Ilmu dan Teknologi Pangan 10(2):212. https://doi.org/10.24843/itepa.2021.v10.i02.p05

Harsa IMS. 2014. Efek pemberian diet tinggi lemak terhadap profil lemak darah tikus putih (*Rattus norvegicus*). Jurnal Ilmiah Kedokteran 31(1):21–28.

Karta IW, Burhannudin B, Kartawidjajaputra F. 2021. Analysis of phytochemical substance and the effect of cang salak tea (Cst) diet to lipid profile on hyperlipidemic rat. Jurnal Sains Dan Teknologi 10(2):239–251. https://doi.org/10.23887/jst-undiksha.v10i2.39302

Kaseb F, Yazdanpanah Z, Biregani AN, Yazdi NB,Yazdanpanah Z. 2018. The effect of chamomile (*Matricaria recutita* L.) infusion on blood glucose, lipid profile and kidney function in type 2 diabetic patients: A randomized clinical trial. Prog Nut 20(1):110–118. https://doi.org/10.23751/pn.v20i1-S.5884

Kothari S, Jain AK, Mehta SC, Tonpay SD. 2011. Hypolipidemic effect of fresh *Triticum aestivum* (wheat) grass juice in hypercholesterolemic rats. Acta Pol Pharm 68(2):291–294.

Kuchta A, Konopacka A, Waleron K, Viapiana A, Wesołowski M, Dąbkowski K, Ćwiklińska A, Mickiewicz A, Śledzińska A, Wieczorek E *et al.* 2021. The effect of *Cistus incanus* herbal tea supplementation on oxidative stress markers and lipid profile in healthy adults. Cardiol J 28(4):534–542.https://doi.org/10.5603/CJ.a2019.0028

Maheshwari V. 2020. Phytochemicals effective in lowering low-density lipoproteins. J Biol Eng Res Rev 7(1):16–23.

Martini R, Rahma A, Kusharto CM, Riyadi H, Sumantri C, Rohdiana D. 2019. The Potential of white tea (*Camellia sinensis*) and kelor (*Moringa oleifera*) in improving lipid profile and histopathological features of pancreas in streptozotocin-induced rats. J Gizi Pangan 14(1):23–30. https://doi.org/10.25182/jgp.2019.14.1.23-30

Millar CL, Duclos Q, Blesso CN. 2017. Effects of dietary flavonoids on reverse cholesterol transport, HDL metabolism, and HDL function. Adv Nutr 8(2):226–239. https://doi.org/10.3945/an.116.014050

Molyneux P. 2004. The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating anti-oxidant activity. Songklanakarin J Sci Technol 26(2):211–219.

Nair R, Ghakker N, Sharma A. 2015. Spectrophotometric estimation of tannins in raw and processed form (*Paan nasala*)

of areca nut. Int J Educ Sci Res Rev 1:51–56.

Nie J, Zhang L, Zhao G, Du X. 2019. Quercetin reduces atherosclerotic lesions by altering the gut microbiota and reducing atherogenic lipid metabolites. J Appl Microbiol 127(6):1824–1834. https://doi.org/10.1111/jam.14441

Prabawati SY, Fajriati I. 2019. Analisis lemak sapi dan lemak babi menggunakan gas chromatography (GC) dan fourier transform infra red spectroscophy second derivative (FTIR-2D) untuk autentifikasi halal. Indonesia Journal of Halal 1(2):89. https://doi.org/10.14710/halal.v1i2.4119

Puspawati NM, Prager RH, Perkin MV. 2019. The chemical constituents of tenggulun (*Protium javanicum* Burm F) leaf. Proceedings of the Indonesian Chemical Society 1(1):17–17. https://doi.org/10.34311/pics.2019.01.1.17

Putri KD, Yusasrini NLA, Nocianitri KA. 2021. Pengaruh metode pengolahan terhadap aktivitas antioksidan dan karakteristik teh herbal bubuk daun Afrika (*Vernonia amygdalina* Delile). Jurnal Ilmu Dan Teknologi Pangan 10(1):77. https://doi.org/10.24843/itepa.2021.v10.i01.p08

Ravikumar C. 2014. Review on herbal teas. J Pharm Sci Res 6(5):236–238

Rizki PR, Jayanti RD,Widyaningsih TD. 2015. Effect of herbal tea based green grass leaf for the level of blood and lipid profile of rat wistar hiperglikemia. Jurnal Pangan dan Agroindustri 3(3):803–814

Rohiqi H, Yusasrini NLA, Puspawati GD. 2021. Pengaruh tingkat ketuaan daun terhadap karakteristik teh herbal matcha tenggulun (*Protium javanicum* Burm F.). Jurnal Ilmu Dan Teknologi Pangan 10(3):345. https://doi.org/10.24843/itepa.2021.v10.i03.p03

Sakanaka S, Tachibana Y, Okada Y. 2005. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). Food Chem 89(4):569–575. https://doi.org/10.1016/j.foodchem.2004.03.013

Santos FA, Frota JT, Arruda BR, de Melo TS, da Silva AADCA, Brito GADC, Chaves MH, Rao VS. 2012. Antihyperglycemic and hypolipidemic effects of α,β-amyrin, a triterpenoid mixture from *Protium heptaphyllum* in mice. Lipids Health Dis 11:1–8. https://doi.org/10.1186/1476-511X-11-98

Siasos G, Tousoulis D, Tsigkou V, Kokkou E, Oikonomou E, Vavuranakis M, Basdra EK, Papavassiliou AG, Stefanadis C. 2013. Flavonoids in atherosclerosis: An overview of their mechanisms of action. Curr Med Chem 20(21):2641–2660. https://doi.org/10.2174/0929867311320210003

Simamora ACY, Yusasrini NLA, Putra INK. 2021. Pengaruh jenis pelarut terhadap aktivitas antioksidan ekstrak daun tenggulun (*Protium javanicum* Burm. F) menggunakan metode maserasi. Jurnal Ilmu Dan Teknologi Pangan 10(4):681. https://doi.org/10.24843/itepa.2021.v10.i04.p13

Sobhani M, Farzaei MH, Kiani S, Khodarahmi R. 2021. Immunomodulatory; Anti-inflammatory/antioxidant effects of polyphenols: A comparative review on the parental compounds and their Metabolites. Food Rev Int 37(8):759–811. https://doi.org/10.1080/87559129.2020.1717523

Soliman GA. 2019. Dietary fiber, atherosclerosis, and cardiovascular disease. Nutrients 11(5):1155. https://doi.org/10.3390/nu11051155

Tariq S, Imran M, Mushtaq Z, Asghar N. 2016. Phytopreventive antihypercholesterolmic and antilipidemic perspectives of zedoary (*Curcuma zedoaria* Roscoe.) herbal tea. Lipids Health Dis 15(1):1–10. https://doi.org/10.1186/s12944-016-0210-y

Topuz A, Dinçer C, Torun M, Tontul I, Şahin-Nadeem H, Haznedar A, Özdemir F. 2014. Physicochemical properties of Turkish green tea powder: Effects of shooting period, shading, and clone. Turk J Agric For 38(2):233–241. https://doi.org/10.3906/tar-1307-17

Yusasrini NLA & Permana IDGMP. 2021. Effect of processing methods on the chemical properties of *Protium javanicum* Burm F herbal tea powder. Food Science and Quality Management 109:19–24. https://doi.org/10.7176/FSQM/109-03

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Effect of Maja (*Aegle marmelous*) Leaf Extract and Trigona Honey on Glucosidase Activity Inhibition

Ulfa Purnamasari, Sri Anna Marliyati*, Evy Damayanthi
[1]Departement of Community Nutrition, Faculty of Human Ecology, IPB University, Bogor 16680, Indonesia

ABSTRACT

This study aims to determine the effect of the maja leaf extract and trigona honey on the α-glucosidase enzyme inhibition, antioxidant activity, and phytochemical type of trigona honey. It is a completely randomized study with natural ingredients, which are traditionally used for diabetic treatment in South Sulawesi. These Maja leaf extracts were mixed with raw trigona honey under several proportions: F1 (only containing trigona honey), FII (trigona honey mixed with maja leaves extract in ratio of 1:1), FIII (only containing maja leaves extract), FIV (trigona honey and maja leaves extract in ratio of 2:1) and FV (trigona honey and maja leaves extract in ratio of 1:2). The five formulas were tested for the phytochemical content (flavonoid, alkaloid, tannin, triterpenoid, steroid, saponin, and quinones), antioxidant activity by DPPH method and followed by α-glucosidase enzyme inhibition test. The phytochemical test found that trigona honey only contained flavonoid and tannin compounds, whereas maja leaf extract and its mixture (with trigona honey) obtained a positive result of flavonoid, tannin, steroid, and saponin contents. Meanwhile, the antioxidant activity results are categorized as follows (IC_{50}): FI (2,524 ppm) has a very weak antioxidant activity, FII (196 ppm) has a weak antioxidant activity, FIII (201 ppm) and FIV (225 ppm) have very weak antioxidant activities, FV (147 ppm) has a moderate antioxidant activity. The results of the α-glucosidase inhibition test show that the highest value was in FV (300.74 ppm), then followed by FIII (493.54 ppm), and FIV (847.95 ppm). On the other hand, FI and FII formulas were considered unable to inhibit α-glucosidase enzymes. Therefore, adding Maja leaf extract into the trigone honey might improve its potential use for managing diabetes.

Keywords: α-glucosidase, antioxidants, extract, maja leaves, trigona honey

INTRODUCTION

The number of deaths related to diabetes reached 1.5 million globally in 2012 and keeps climbing. In Indonesia, the prevalence in 2018 has increased by 2 % from 2013 (RISKESDAS 2018). Food intake affects the amount of insulin needed to meet blood glucose target. Carbohydrate in diet affects postprandial blood glucose levels and it is a major determinant of food-related insulin requirements (Tiwari *et al.* 2013). However, once someone has diabetes, medicine is needed to control the blood glucose. The available hypoglycemic agents cause digestive discomfort and might not able to prevent diabetes complication. Thus, the use of natural or herbal ingredients which potentially act as inhibitors of the α-glukosidase enzyme might offer better blood glucose control for people with diabetes (Lisiswanti & Faris *et al.* 2017).

Trigona honey (*Biroi*) and maja leaves (*Aegle marmelos*) are two natural inggridients native from Indonesia which have the potentials for the treatment of degenerative diseases. Although they have been used as traditional medicine (diabetes), but the development using scientific approach has been scarce. Research by Sumarlin *et al.* (2015) on the bioactivity from combination of Trigona honey with the methanol extract of namnam leaves reported that the combination of these two natural ingredients has the potential as an antioxidant and antibacterial substances. Furthermore, a combination of honey and black seed (*Nigella sativa*) is able to accelerate wound healing compared to the single use of each ingredient where its effect is almost similar to the drug phenytoin (Javadi *et al.* 2018).

Trigona honey also possesses bioactive compounds that potentially act as anti-diabetic substance according to Ali *et al.* (2020). Trigona

*Corresponding Author: tel: +628121105760, email: marliyati@apps.ipb.ac.id
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honey called as *Kelulut* or Tualang honey in Malay produced by *Heterotrigona itama* bee contains phenolic and flavonoids compounds that are able to inhibit the activitiy α-amylase and α-glukosidase enzymes. Flavonoids protect cell from damage and re-stimulate insulin receptor sensitivity in order to produce insulin (Susanti *et al.* 2020). The α-glukosidase enzyme, which is present in the mucosa of the small intestine, breaks down disaccharides into simple sugars, previously in the form of complex polysaccharides that get broken down by α-amylase enzyme (Febrinda 2013).

Fruit of maja plant can be eaten directly, while the leaves and shoots are consumed as vegetables or salads in various countries in Asia. In Indonesia, they are consumed as cooking spices and used to reduce appetite (Nigam & Nambiar 2015). Maja plants contain many phytochemicals such as carotenoids, phenolics, alkaloids, pectin, tannins, coumarins, flavonoids, and terpenoids (Manandhar *et al.* 2018). Research by Mudi *et al.* (2017) showed that the glucose-lowering effect of the Maja leaf and fruit extracts were related to the effect of increased insulin sensitivity by lowering insulin resistance in type 2 diabetic rats.

Research on the mixing of trigona honey and maja leaf exract aims to determine the effect of Maja (*Aegle marmelous*) leaf extract and proportion of trigona honey on inhibition of glucosidase activity as antidiabetes. It is expected to produce a positive synergistic effect on the content of phytochemical compounds, antioxidant activity, and α-glucosidase enzyme inhibitory activity. Therefore, this study is expected to provide some information related to the antioxidant and antidiabetic activity of mixed in trigona honey and maja leaf extract.

METHODS

Design, location, and time

This study used a completely randomized design. The study involved several stages and test. The research samples, maja leaves and stingless bee honey (raw), were studied at two different locations. The extraction and antioxidant activity test were conducted in the biopharmaceutical laboratory at Hasanudin University. Then, the phytochemical and α-glucosidase enzyme inhibitory analysis of the sample was analyzed in biopharmaceutical laboratory of IPB University. The samples were sent in amber screw bottle to prevent antioxidant damage with normal temperature from Hasandin University to IPB University. The study was conducted from September to November 2021.

Material and tools

The main materials for this study were maja leaves (*Aegle marmelous*) from Makassar and stingless bee honey (*Trigona biroi* sp) from Bone-Bone village, North Luwu of South Sulawesi. These materials were processed with 96% ethanol solvent, filter paper, hot water, 0.05 Mg powder, concentrated HCl 1 ml, amyl alcohol, glacial CH_3COOH, concentrated H_2SO_4, 10 ml of chloroform, ammonia, H_2SO_4 2M; Dragendorf, Meyer, and Wagner reagents; distilled water, HCl 1N, $FeCl_3$ 1%, 0.002% DPPH 6 ml, 0.004% DPPH 12 ml, methanol, ascorbic acid, p-nitrophenyl-D-glucopyranoside, 0.1M phosphate buffer pH 7.0, α-glucosidase, dimethyl sufoxide (DMSO), 1% acarbose solution, and 2 NHcl.

The instruments or tools used in this study included a rotary evaporator (Buchi R220, Germany), blenders (Signora, Indonesia), hot plates (Thermo SP 131320-33q, USA), dropper pipettes, digital scales (Denver instrument SI-234, USA), spectrophotometer (Shimadzu 1800, Japan), microplates, and microplate readers (biotek ELx808, USA).

Procedures

Extraction of maja leaves and phytochemical testing. Seven hundred grams of fresh maja leaves was put in an oven and dried at temperature of 50°C for 8 h to obtain 100 g of dried maja leaves. These were observed every 2 h. Next, these dried leaves were grounded into fine powder (simplisia) using a simplisia grinding machine (memmert, USA) with water content <10% (Sumarlin *et al.* 2015). These simplisia powder was then soaked with 96% ethanol and put into maceration process for 24 h. After the process finished, the sample was filtered to obtain the first filtrate result (Edison *et al.* 2020). Then, the maja leaves residue was macerated again with ethanol solvent for 9 h, in order to obtain the second filtrate result. As the last step, the filtare was evaporated by using a rotary evaporator at 49°C with pressure 50 bar for 5 h to obtain a thick extract as much as 31 g which was used in the experiment with ethanol content of the extract not exceeding 1% v/v (20°C) (Sumarlin *et al.* 2015).

Formula determination. The formula for this study was created from maja leaf extract and trigona honey. The ratio used was based on a comparison of the content of active compounds contained in trigona honey and maja leaf extract, which has an antidiabetic potential. Research by Ali *et al.* (2020) stated that from honey concentration of 80 g/ml and 100 g/ml, there was an inhibition of α- glukosidase enzyme of more than 50% in Trigona Itama honey. The most common feed for these bees are mangrove, multi-fruits and *tualang*. In line with this research, (Phuwapraisirisan *et al.* 2008) succeded in isolating new active compounds called anhydroaegelin, aegelinoside A and aegelinoside B, where the most potential compound for inhibiting the α-glukosidase enzyme was anhydroaegelin, under a concentration of 10 g/ml with an IC_{50} inhibitory value of 35.8 M by using an 0.5 mg/ml extract. The LD50 for maja leaf extract was 10 g/kg (Phuwapraisirisan *et al.* 2008). The researchers concluded that the required materials for testing the inhibition of α-glukosidase enzyme were 100 g/ml or equivalent to 0.1 mg/ml of trigona honey and 500 g/ml or equivalent to 0.5 mg/ml of maja leaves extract as seen in Table 1.

Test of flavonoid content. A total of 50 mg sample was weighed then added with 100 ml of hot water and boiled for about 5 min, and then filtered to obtain 5 ml filtrate. The powder 0.05 mg of Mg, 1 ml of concentrated HCl, and amyl alcohol was added to the produced filtrate. The sample created different colours such as red, yellow, or orange on the amyl alcohol layer if the test had a positive result (Edison *et al.* 2020).

Table 1. Proportions of mixed trigona honey and maja leaves formula used in this study

Formula	Trigona honey (raw) (mg/ml)	Maja leaves extract (mg/ml)
I (1:0)	0.1	-
II (1:1)	0.1	0.5
III (0:1)	-	0.5
IV (2:1)	0.2	0.5
V (1:2)	0.1	1.0

The proportion of comparisons is based on studies that have succeeded in obtaining a positive effect on the alpha-glucosidase enzyme test namely

Ali *et al.* (2020) using 0.1 mg/ml trigona honey and Phuwapraisirisan *et al.* (2008) using Maja leaves extract 0.5 mg/ml

Test of triterpenoid/steroid content. A total of 50 mg sample was weighed then added with 10 drops of glacial CH_3COOH and 2 drops of concentrated H_2SO_4. This solution was then shaken slowly and let for several minutes. The sample presented particular colors with red or purple colors indicated triterpenoids content while blue or green colors indicated steroid content (Edison *et al.* 2020).

Test of saponin content. A total sample of 50 mg was weighed and added with 10 ml water and shaken for 1 min then added with 2 drops of 1 N HCL. The saponin content was indicated by the foam formation that remained stable for 7 min (Edison *et al.* 2020).

Test of tannin content. A total of 50 mg sample was weighed and added with 10 drops of 1 % $FeCl_3$. Then, an observation was done where the sample produced green, red, purple, blue or solid black colours when the sample had positive result (having tannin content) (Edison *et al.* 2020).

Test of quinon content. A total of 50 mg sample was weighed and added with 2 drops of 1 M NaOH. Then, the sample produced red colour which indicated that there was a positive result (having quinon content) (Edison *et al.* 2020).

Antioxidant determination by DPPH methods (Sumarlin et al. 2015). A total of 2 ml sample was put into a test tube then added with 2 ml of 0.002 % DPPH. Next, the samples were homogenized and incubated for 30 min in a dark room and absorption was read at 517 nm with a UV-VIS spectrophotometer. The measurement was repeated three times. For standard curve ascorbic acid standard was prepared at a concentration of 0.5, 1, 2, 4, and 8 ppm with the same treatment as the test sample. The absorbance value obtained was then used to determine the percentage of inhibition value. Then, the result from calculations was entered into a regression calculation where IC_{50} value (Inhibition Concentration) was obtained when the percent inhibition value was 50% (Sumarlin *et al.* 2015).

Inhibitor α-glukosidase test (Widowati et al. 2015). The first solution to be reacted was Control Blank Solution (B0), Blank Solution (B1), Control Sample Solution (S0), and Sample Solution (S1). The substrate preparation was made by dissolving p-nitrophenyl α-D-glucopyranoside in 0.1 M phosphate buffer (pH 7.0). Meanwhile, the α-glucosidase enzyme solution was made by dissolving 1 mg of α-glucoside in 100 ml of phosphate buffer (pH 7.0). The blank solution consisted of 10 µl of Dimethyl sulfoxide (DMSO) solution, 50 µl of 0.1 M phosphate buffer (pH 7.0), 25 µl of p-nitrophenyl α-D-glucopyranose acted as substrate, and 25 µl of -glucosidase enzyme solution. The difference between the blank and the control blank was that the blank control did not use the α-glucosidase enzyme.

Preparation of Sample Control Solution (S0) and Sample Solution (S1) was carried out by dissolving maja leaf extract and trigona honey in buffer. The sample reaction mixture consisted of 10 µl extract, 50 µl 0.1 M phosphate buffer (pH 7.0), 25 µl p-nitrophenyl -αD-glucopyranoside 0.5 mM as a substrate, and 25 µl of α-glucosidase enzyme solution. The difference between the sample and the control sample is that the control sample did not use the α-glucosidase enzyme.

The positive control applied in this test was acarbose with concentration of 1%. A sample of comparison was made from Glucobay tablet dissolved in distilled water and 2 NHcl (ratio of 1:1) under concentration of 1% (w/v) and centrifuged. A total of added 10 µl of supernatant was taken and added into the samples. Each reaction sample was mixed into different microplates and the solution on the microplate was incubated at 37°C for 30 min.

As the next phase, the solution was added with Na_2CO_3 to stop the reaction and the inhibitory activity was read with a microplate reader at 410 nm. The inhibitory power of the extract was calculated as percentage (%) of inhibition as follows:

$$Inhibition(\%) = [(K - (S1 - S0)/K)]\ X\ 100\%$$

Where:
K: Absorbance of Blank (B1) - Absorbance of Blank (B0) = Absorbance of Control Sample (S0)
S1: Absorbance of Sample (Sumarlin *et al.* 2015).

Data analysis

The data collected were compiled with Microsoft Excel 2010 and qualitative phytochemical test results are presented in descriptive analysis. The antioxidant activity and α-glucosidase enzyme inhibition tests were analyzed using regression formula in Microsoft Excel 2010 for the IC_{50} value.

RESULTS AND DISCUSSION

Phytochemical test

There are phytochemical compounds found in plants that are able to counteract free radicals, such as phenolic, flavonoids, coumarin derivatives and other compounds (Febrinda *et al.* 2013).

As shown in Table 2, flavonoid was identified in trigona honey, maja leafs extract and mixture of both materials. Most green plants have a high flavonoid content compared to non green plants (Widotiasari 2016). In addition, Ali *et al.* (2020) reported that trigona honey has flavonoid compounds including trigona honey from bees of *Itama* spp. Flavonoids act as antioxidants, inhibitors of α-glucosidase enzymes, and antibacterials substance. A similar study was proposed by Ningrum *et al.* (2019) who also obtained a positive test result for flavonoid content in maja leaves. Flavonoids have been shown to be a powerful agent to reduce the pathogenesis of diabetes and its complications. The antidiabetic mechanism of flavonoids was to reduce apoptosis and insulin resistence and increase insulin secretion and GLUT 4 translocation. Regular consumption of cyanidin inhibits glucosidase and amylase, reducing the absorption of carbohydrates in the intestine (Al-Ishaq *et al.* 2019). Alkaloid compounds can also act as an antioxidant (Sulasiyah 2018). However, there was no identified alkaloid compound content in all formulas.

Meanwhile, for tannin compound, all formulas produced a positive reaction. Tannins are among phenolic compounds that give bitter and astringent/chelate taste and they are able to aggromerate protein, amino acid and alkaloids (Julianto 2019). Tannins from plants are well known antioxidants. Tannins enhance glucose uptake and inhibit adipogenesis, thus having potentials for treatment of diabetes type 2 (Kumari & Jain 2012).

The content of triterpenoid was not found in this study because the samples did not produce blue colour, instead it produced green colour as an indication of a positive result of steroid content. Research by Sumarlin *et al.* (2018) that tested triterpenoid content in honey and its combination with namnam leaves also did not produce a positive result of triterpenoid only in honey, but it had positive result in mixture of namnam leaves and honey. The green colour was formed when the sample was treated with glacial CH_3COOH and concentrated H_2S0_4 in formulas of FII, FIII, FIV and FV, which indicated a positive result for steroid content. As for the F1 formula that contained only trigona honey, it did not indicate any steroid content. Thus, the addition of maja leafs extract provided a strong steroid content in the formulas. This result aligned with the research of Sumarlin *et al.* (2018) where the steroid content in rambutan honey was not found in the honey sample only but it was apparent in namnam leaf extract and in the mixture of namnam leaf extract with rambutan honey. Steroids are part of saponin family namely sapogenins, which is an antioxidant known to have an effect on the treatment of diabetes (Astuti *et al.* 2012).

There was no saponin content in the F1 with only trigona honey. Saponin was only found in maja leaf extract. Hence, the formulas with maja leaf extract (FIII, FIV, and FV) were able to produce a positive test of saponin. Saponin, as a glucosidase enzyme inhibitor, inhibits the breakdown of carbohydrates into glucose. Saponins works through regeneration of pancreas, which causes an increased number of pancreatic β-cells and the Langerhans Island, so that insulin secretion can help reduce blood glucose levels. The regeneration of pancreatic β-cells occurs due to the presence of quiescent cells in the pancreas, which have ability to regenerate (Susanti *et al.* 2020). Saponin, which gives an effect in the bubble formation, can cause hemolysis in red blood cells. Another example of saponin glycosides is liquorice, with its expectorant and anti-inflammatory activities, or diosgin compound, which is important for formation of glucocorticoid and for steroid hormones as progesterone (Julianto 2019). Due to the content of saponin in maja leaf extract, the leaves are known and used as a traditional anti-fertility agent (Vinita Bisht 2017).

Quinone content was identified by red colour formation in sample treated with 1 M NaOH. None of the formulas formed a red colour, whether in trigona honey sample, maja leaf extract, or in the mixture of both. Research of Sumarlin *et al.* (2018) also obtained the same results where there was an absence of quinone

Table 2. Result of phytochemical test on mixtures of maja leaf ethanol extract with trigona honey

Test result	Formula				
	FI	FII	FIII	FIV	FV
Flavonoid	+	+	+	+	+
Alkaloid	-	-	-	-	-
Tanin	+	+	+	+	+
Triterpenoid	-	-	-	-	-
Steroid	-	+	+	+	+
Saponin	-	+	+	+	+
Quinon	-	-	-	-	-

FI: Trigona honey (raw) (1:0)
FII: Trigona honey (raw): Maja leaves extract (1:1)
FIII: Maja leaves extract (0:1)
FIV: Trigona honey (raw): Maja leaves extract (2:1)
FV: Trigona honey (raw): Maja leaves extract (1:2)

content in rambutan honey mixed with namnam leaves extract. Phytochemical compound in honey is influenced by the dominant type of plants consumed by the bees.

Antioxidant activity test

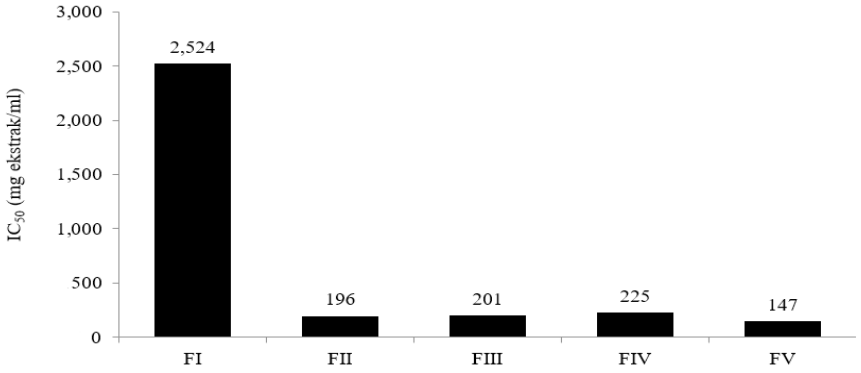
The antioxidant activity was determined by IC_{50} (inhibition concentration) value as it indicates the ability of the extract to inhibit free radicals' activity (2,2-diphenyl-1-picryhydrazyl or DPPH) that is marked by colour change in the solution from light purple to yellow to mark the oxidation of free radicals. The color change was then measured quantitatively to obtain absorption used to find the IC_{50} value. The IC_{50} value was obtained by employing a linear regression formula, which stated the relationship between antioxidant fraction concentration (x) and percent of inhibition (y) (Purwanto *et al.* 2017).

The IC_{50} value in antioxidant activity is divided into five categories; the IC_{50} value of less than 0.05 mg/ml (<50 ppm) as a very strong antioxidant group, the IC_{50} value in the range of 0.05–0.1 mg/ml (50–100 ppm) as a strong antioxidant group, the IC_{50} value in the range of 0.1–0.15 mg/ml (100–150 ppm) as a moderate antioxidant group, the IC_{50} value in the range of 0.15–0.2 mg/ml (150–200 ppm) as a weak antioxidant group,and the IC_{50} value in the range of more than 0.2 mg/ml (>200 ppm) as a very weak antioxidant group (Susana *et al.* 2018).

Figure 1 shows that F1 obtained IC_{50} value of 2,524 ppm, which means that the antioxidant activity of sample trigona honey was very weak; meanwhile, only FIV (a mixture with extra/more trigona honey than the maja leaf extract) had IC_{50} value of 225 ppm and FIII (maja leaves extract without a mixture) had IC_{50} value of 201 ppm, which means that the antioxidant activity found in the two formulas were in very weak. Stronger antioxidant activity was found in FII (a mixture of trigona honey and maja leaf extract in equal ratio) with IC_{50} value of 196 ppm. Meanwhile, the FV (a mixture of trigona honey and maja leaf extract with a higher ratio of maja leaf extract) had IC_{50} value of 147 ppm and it was the moderate category.

Muruke's reseach (2014) found that antioxidant activity of trigona honey was 4.19 mg/ml or 4.190 ppm. Meanwhile, the higher antioxidant activity from maja leaf extract was in accordance with Wilujeng *et al.*'s (2020) research that found a strong antioxidant activity in maja fruit extract with IC_{50} value of 0.107µg/ml.

The formulas with potential antioxidant (<200 ppm) in the study were found in FII and FV that showed addition of maja leaf extract to trigona honey gave a positive effect to the antioxidant activity. This result also aligned with the previous phytochemical test result in all formulas added with maja leaf extract that were found to have various phytochemical compounds. Antioxidants can stop the free radical auto oxidation in lipid oxidation (Susana *et al.* 2018). Increased free radicals activity is associated with various diseases, such as diabetes, cancer, cardiovascular diseases or other degenerative diseases. Degenerative disease such as diabetes



FI: Trigona honey (raw) (1:0)
FII: Trigona honey (raw): Maja leaves extract (1:1)
FIII: Maja leaves extract (0:1)
FIV: Trigona honey (raw): Maja leaves extract (2:1)
FV: Trigona honey (raw): Maja leaves extract (1:2)

Figure 1. The result of IC_{50} test of antioxidant activity from maja leaves ethanol extract and trigona honey

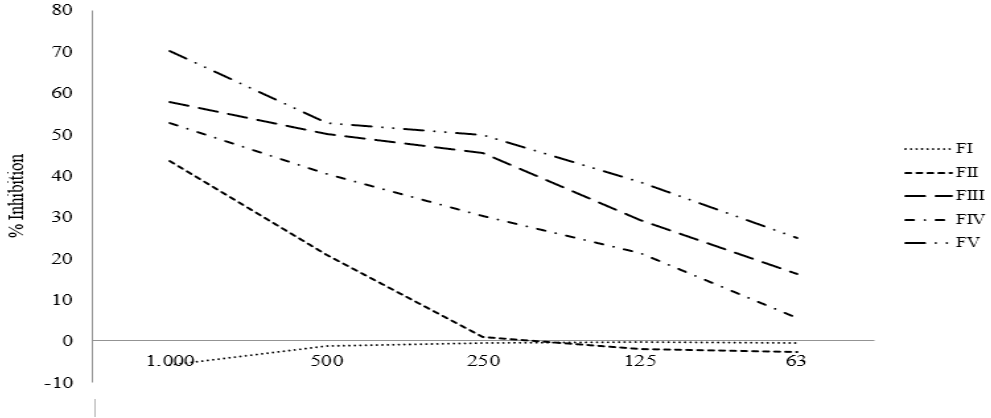
can be caused by the presence of reactive and unstable free radicals that attack macromolecules causing oxidative stress in mitochondria which will stimulate glucose intolerance. Meanwhile, the state of glucose intolerance in diabetics can result in production of free radicals, characterized by inflammatory oxidation and increased activity of (NAD(P)H oxidase that induces the formation of reactive free radicals. Free radicals increase the expression of TNF- (Tumor Necrosis Factor) and exacerbate stress. Increased oxidative stress, TNF- and cytokines, such as interleukins, can cause insulin resistance by decreasing insulin receptor autophosphorylation (Husain & Kumar 2012).

Inhibition α-glucosidase test

The α-glucosidase inhibition test was employed to determine the anti-diabetic effect from trigona honey, maja leaves extract, and their mixture in several different proportions. In vitro analysis of α-glucosidase enzymes tests the sample's inhibition reaction against p-nitrophenyl α-D-glucopyranosia substrate. If the tested sample has the ability to inhibit the α-glucosidase enzyme, then the resulting p-nitrophenyl will be reduced (Pratama *et al.* 2015).

In the p-nitrophenyl test, the formula turns into yellow colour when the α-glucoside enzyme hydrolyzes the substrate. The absorption of the resulted color is measured by a spectrophotometer and used to calculate the inhibition percentage (%) and then applied for IC_{50} value determination.

The results showed that the inhibition percentages were between -5.85 until 43.64% inhibition in all samples. It indicated that FI and FII formulas did not have potentials as to inhibit α-glucosidase enzymes. Meanwhile, FIII, FIV and FV were proven to have inhibition percentages above 0%. So, these results could be used to calculate IC_{50} which indicated their potential ability as an inhibitor of α-glucosidase enzyme. The research from Gurudeeban *et al.* (2012) suggested a similar result where the average inhibition percentage of maja leaves and fruit extract ranged from 72.23±0.30. In this assay, FV (a mixture of trigona honey and maja leaf extract with a higher percentage of maja leaf extract) displayed the highest α-glucosidase inhibition. This probably related to the phytochemical contents of maja leaf as recorded in this study. Similar inhibition activity of α-glucosidase enzyme in trigona honey was also shown by Rahmawati *et al.* (2019) and Ali *et al.* (2020) with the value of IC_{50} 1.917 ppm with 74.82±2.39 % inhibition. This result showed that an increase in maja leaf extract proportion added positive effect to the phytochemical content, antioxidant activity and α-glucosidase enzyme inhibitory activity in the formulas (Figure 2).



Values are presented in average value (n=3)
FI: Trigona honey (raw) (1:0)
FII: Trigona honey (raw): Maja leaves extract (1:1)
FIII: Maja leaves extract (0:1)
FIV: Trigona honey (raw): Maja leaves extract (2:1)
FV: Trigona honey (raw): Maja leaves extract (1:2).

Figure 2. The result of inhibitory α-glucosidase test

CONCLUSION

Phytochemical, antioxidant activity and α-glucosidase enzyme inhibition tests showed that the addition of maja leaf extract to trigona honey in increasing proportion were beneficial to the antioxidant activity and α-glucosidase enzyme inhibition. The addition of maja leaf extract was able to produce positive effect to the phytochemical content (flavonoids, tannins, steroids, and saponins compounds). Meanwhile, trigona honey was found to only contain alkaloids and tannins. Moreover, the antioxidant activity and α-glucosidase enzyme inhibitory activity of the formula also increased along with the addition of maja leaf extract. Further research to test more variations of proportions for obtaining a more precise dose so that it can be used for preventing degenerative diseases, such as diabetes. Therefore, based on this in-vitro study, we recommend that mixing of trigona honey and maja leaf extract may offer beneficial effects for managing diabetes.

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DECLARATION OF INTERESTS

The authors declare no conflict of interest with any party, other person or institution for this research.

REFERENCES

Al-Ishaq RK, Abotaleb M, Kubatka P, Kajo K, Büsselberg D 2019. Flavonoids and their anti-diabetic effects: Cellular mechanisms and effects to improve blood sugar levels. Biomolecules 9(9):430–465. https://doi.org/10.3390/biom9090430

Ali H, Abu Bakar MF, Majid M, Muhammad N, Lim SY. 2020. In vitro anti diabetic activity of stingless bee honey from different botanical origins. Food Res 4(5):1421–1426. https://doi.org/10.26656/fr.2017.4(5).411

Astuti SM, Sakinah AM, Risch A. 2012. The Triterpenoid Saponin from Binahong [Anredera Cordifolia (Ten) Steenis] to Potential Using as Antidiabeticactivity in Animal Laboratory. Proceeding of International Conference on Drug Development of Natural Resources (page 331–343), 30th June. Yogyakarta (ID): Universitas Ahmad Dahlan.

Edison E, Diharmi A, Ariani NM, Ilza M 2020. Komponen bioaktif dan aktivitas antioksidan ekstrak kasar Sargassum plagyophyllum. J Pengolah Has Perikan Indones 23(1):58–66. https://doi.org/10.17844/jphpi.v23i1.30725

Febrinda AE, Astawan M, Wresdiyati T, Yuliana ND. 2013. Kapasitas antioksidan dan inhibitor alfa glukosidase ekstrak umbi bawang dayak. Jurnal Teknologi dan Industri Pangan 24(2):161–161. https://doi.org/10.6066/jtip.2013.24.2.161

Gurudeeban S, Satyavani K, Ramanathan T. 2012. Alpha-glucosidase inhibitory effect and enzyme kinetics of coastal medicinal plants. Bangladesh J Pharmacol 7(3):186–191. https://doi.org/10.3329/bjp.v7i3.11499

Husain N, Kumar A. 2012. Reactive oxygen species and natural antioxidants: A review. Adv Biores 3(4):164–175.

Javadi SMR, Hashemi M, Mohammadi Y, MamMohammadi A, Sharifi A, Makarchian HR. 2018. Synergistic effect of honey and nigella sativa on wound healing in rats. Acta Cir Bras 33(6):518–523. https://doi.org/10.1590/s0102-865020180060000006

Julianto TS. 2019. Fitokimia Tinjauan Metabolit Sekunder dan Skrining Fitokimia. Yogyakarta (ID): Penerbit Universitas Islam Indonesia.

Kumari M, Jain S. 2012. Tannins: An antinutrient with positive effect to manage diabetes. Res J Recent Sci 1(12):70–73.

Lisiswanti R, Haryanto FP. 2017. Allicin in garlic (*Allium sativum*) as alternative therapy type 2 diabetes mellitus. Majority 6(2):33–38.

Manandhar B, Paudel KR, Sharma B, Karki R. 2018. Phytochemical profile and pharmacological activity of Aegle marmelos Linn. J Integr Med 16(3):153–163. https://doi.org/10.1016/j.joim.2018.04.007

Mudi SR, Akhter M, Biswas SK, Muttalib MA, Choudhury S, Rokeya B, Ali L. 2017. Effect

of aqueous extract of Aegle marmelos fruit and leaf on glycemic, insulinemic and lipidemic status of type 2 diabetic model rats. J Complement Integr Med 14(2). https://doi.org/10.1515/jcim-2016-0111

Muruke MH. 2014. Assessment of antioxidant properties of honeys from Tanzania. Journal of Biology, Agriculture and Healthcare 4(27):22–32.

Nigam V, Nambiar VS. 2015. Therapeutic potential of *Aegle marmelos* (L.) Correa leaves as an antioxidant and anti-diabetic agent: A review. Int J Pharm Sci Res 6(3):611–621.

Ningrum HTR, Hidayah DR, Larassati F, Wisanti W. 2019. Efektivitas ekstrak daun maja (*Crescentia cujete* L.) sebagai antibakteri pada bakteri E. coli dan S. aureus. Proceeding Biology Education Conference 16(1):285–287.

Phuwapraisirisan P, Puksasook T, Jong-Aramruang J, Kokpol U. 2008. Phenylethyl cinnamides: A new series of α-glucosidase inhibitors from the leaves of *Aegle marmelos*. Bioorganic Med Chem Lett 18(18):4956–4958. https://doi.org/10.1016/j.bmcl.2008.08.024

Pratama Y, Sarjono PR, Mulyani NS. 2015. Skrining metabolit sekunder bakteri endofit yang berfungsi sebagai antidiabetes dari daun mimba (*Azadirachta indica)*. J Kim Sains Apl 18(2):73–78. https://doi.org/10.14710/jksa.18.2.73-78

Purwanto D, Bahri S, Ridhay A. 2017. Uji aktivitas antioksidan ekstrak buah purnajiwa (*Kopsia arborea* Blume) dengan berbagai pelarut. J Ris Kim 3(1):24–32. https://doi.org/10.22487/j24775398.2017.v3.i1.8230

[RISKESDAS] Riset Kesehatan Dasar. Center of Data and Information Indonesia Ministry of Health. 2018. Hasil Utama Riskesdas 2018. Jakarta (ID): Ministry of Health Republic of Indonesia

Rahmawati O, Pratami DK, Raffiudin R, Sahlan M. 2019. Alpha-glucosidase inhibitory activity of stingless bee honey from tetragonula biroi and tetragonula laeviceps. AIP Conf Proc 2092(1):030001 . https://doi.org/10.1063/1.5096705

Singh B, Sharma RA. 2020. Secondary Metabolites of Medicinal Plants: Ethnopharmacological Properties, Niological Activity and Production Strategis. Germany (DE): John Wiley & Sons.

Sulasiyah, Sarjono PR, Aminin AL 2018. Antioxidant from turmeric fermentation products (*Curcuma longa*) by Aspergillus oryzae. J Kim Sains Apl 21(1):13–18. https://doi.org/10.14710/jksa.21.1.13-18

Sumarlin LO, Suprayogi A, Rahminiwati M, Tjahja A, Sukandar D. 2015. Bioaktivitas ekstrak metanol daun namnam serta kombinasinya dengan madu trigona. Jurnal Teknologi dan Industri Pangan 26(2):144–154. https://doi.org/10.6066/jtip.2015.26.2.144

Sumarlin LO, Chalid SY, Sukandar D. 2018. Aktivitas antioksidan kombinasi madu monoflora dengan ekstrak daun namnam (*cynometra cauliflora* L.). Alchemy 6(1):10–17. https://doi.org/10.18860/al.v6i1.4736

Susana I, Ridhay A, Bahri S. 2018. Kajian aktivitas antioksidan ekstrak batang kecombrang (*Etlingera elatior*) berdasarkan tingkat kepolaran pelarut. J Ris Kim 4(1):16–23. https://doi.org/10.22487/kovalen.2018.v4.i1.10178

Susanti Y, Adriani M, Adi AC. 2020. Effect of single clove garlic extract (*Allium sativum* Linn) on blood sugar levels, Malondialdehyde, insulin levels and insulin resistance (Experiments in rats (*Rattus novergicus*) induced by streptozotocin. Strada Jurnal Ilmiah Kesehatan 9(2):954–963.https://doi.org/10.30994/sjik.v9i2.411

Tiwari IBK, Pandey KB, Abidi AB, Rizvi SI. 2013. Markers of oxidative stress during diabetes mellitus. J. Biomark 2013(378790):1–8. https://doi.org/10.1155/2013/378790

Vinita Bisht N, Johar V. 2017. Bael (*Aegle marmelos*) Extraordinary Species of India: A Review. Int J Curr Microbiol App Sci 6(3):1870–1887. https://doi.org/10.20546/ijcmas.2017.603.213

Widotiasari IP. 2016. Pengetahuan tentang manfaat kesehatan, aktivitas inhibisi enzim α-glukosidase, dan uji toksisitas minuman binahong (*Anredera cordifolia*) [Thesis]. Bogor: Institut Pertanian Bogor.

Widowati W, Maesaroh M, Fauziah N, Erawijantari PP, Sandra, F. 2015. Free

radical scavenging and α-/β-glucosidase inhibitory activities of rambutan (*Nephelium lappaceum* L.) peel extract. Indones Biomed J 7(3):157–162. https://doi.org/10.18585/inabj.v7i3.180

Wilujeng S, Suharnanik S, Tiaranisa P. 2020. Antioxidant maja fruit (*Aegle marmelos* (L) carrea) lowering blood sugar mus musculus. Bp Int Res Exact Sci 2(3):362–367. https://doi.org/10.33258/birex.v2i3.1075

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Knowledge, Attitude and Practices of Fruit Consumption among Female and Male Adolescents in Hulu Terengganu and Marang, Malaysia

Nurul Atikah Jamaludin, Mohd Adzim Khalili Rohin, Norhayati Binti Abd Hadi*
School of Nutrition & Dietetics, Faculty of Health Sciences, Universiti Sultan Zainal Abidin, Gong Badak Campus, 21300 Kuala Nerus, Terengganu Darul Iman, Malaysia

ABSTRACT

The study aimed to determine the level of knowledge, attitude and practice of fruit consumption and their associations among female and male adolescents in Hulu Terengganu and Marang. This a cross-sectional study involving 184 selected secondary school students in Hulu Terengganu and Marang, with 94 of them were female respondents and 90 were male. Data on socio-demographic, fruit consumption, knowledge, attitude and practices of fruit consumption were collected using validated questionnaire and food frequency questionnaire. Only 35.8 % of the respondents had adequate fruit consumption, 62.8% and 60.0% of female and male respondents had good knowledge, 63.8% and 60.0% of female and male respondents had good attitude and only 6.4% and 16.7% of female and male respondents had good practice. We also found a significant difference of practice in fruit consumption between male and female respondents ($p<0.05$). The result showed that there was positive significant correlations between knowledge and attitude as well as attitude and practices of fruit consumption ($p<0.05$). No correlation was reported between knowledge and practices of fruit intake. This showed that good attitude potentially improved knowledge and practice towards fruit consumption and vice versa. The results of this research showed that it is crucial to improve the main factors to influencie knowledge, attitude and practices of fruit consumption among adolescents.

Keywords: attitude, fruit, knowledge, practice

INTRODUCTION

World Health Organization reported that almost one fifth of adolescents aged 10−19 years old have a nutritional problem which affect their well-being and livelihood in later life (WHO 2014). Inadequate consumption of fruits contributed about 2.8 % of mortality cases of non-communicable diseases such as gastrointestinal cancer, heart disease and diabetes (WHO 2014). More than one third of adolescents was overweight or obese which related to low fruit consumption (Boeing *et al.* 2012). Since higher fruit preferences among adolescents was recommended for good dietary intake in later life, increasing the fruit intake is an important issue among adolescents nowadays (Craigie *et al*. 2011).

Fruits have been recognized as a nutritious food sources due to its health promoting agents with various micronutrients and phytochemical compounds (Liu 2013). The minimum fruit recommendation for adolescent is two servings daily, which approximately equivalent to 160 g of fruit (WHO 2003; Institute for Public Health (IPH) 2013). Despite its beneficial effect for human, fruit intake among adolescents worldwide is low. Only 31.5% of adolescents achieved the recommended intake where rural population contributed the higher percentage (IPH 2017). Among primary and secondary school students in Malaysia the proportion of students who prefer fruits as their snacks were 25.0% and 17.7%. Thus, in Malaysia, some strategies are taken to improve accessibility of high quality of fruits at the retail stores, expanding farm-to-institution practices in schools, workplaces or hospitals as well as empowerment of policies to improve activities regarding fruit and vegetables in schools (IPH 2017).

Fruit consumption among adolescents are associated with the knowledge, attitude and practices of fruit consumption (Hassan *et al.* 2015). However, to the best of our knowledge, limited data were available on knowledge, attitude and practice of fruit consumption among

***Corresponding Author:** tel: +6096688586, email: norhayatihadi@unisza.edu.my
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adolescents in rural areas in Asian countries (Harrington 2016; Ham & Kim 2014).

The understanding on the association of Knowledge, Attitude and Practices (KAP) studies of fruit consumption may provide ideas in creating effective interventions to improve fruit consumption among adolescents which can lead to reducing the increasing risk of malnutrition (Rojroongwasinkul *et al.* 2013; Poh *et al.* 2013). Risk of malnutrition is high among the low socio-economy status group; thus, by focusing on the rural area, the study will help guide public policies in developing healthy environment, which is suitable to this demography (Menezes *et al.* 2017). Therefore, the current study is aimed to assess the current KAP of fruit consumption among adolescents in rural area. Since minimal research was done in rural areas, thus this study will be focusing in the Hulu Terengganu and Marang areas to represents the rural Terengganu, Malaysia.

METHODS

Design, location, and time

A cross-sectional study was carried out to determine the knowledge, attitude and practices of fruit consumption among female and male secondary school students in Rural Terengganu. Ethical approval for the study was received on 1st January 2019, with the reference number UNISZA/UHREC/2018/66.

Sampling

Convenience purposive sampling method was used in this study. List of secondary school in Marang and Hulu Terengganu were obtained from the District Education Office. A total of four schools from the two selected districts with eight classes, whose students aged 14 and 16 years old, provided a total sample of 184 respondents. The respondents were chosen based on the inclusion and exclusion criteria.

The eligibility criteria were: 1) Age 14 and 16 years old, were not taking any Malaysian Public examination; 2) School is located in rural Terengganu; 3) Willing to take part in the study; 4) Able to read, write and understand questionnaire. Students with acute or severe diseases, special need children, international students and those who were unable to understand the questionnaire were excluded. The chosen respondents completed the questionnaire with informed consent and briefing given prior to the data collection.

Randomization of sampling method. The list of the secondary schools in Terengganu were obtained form the Ministry of Education: 1) Stage 1: Two out of eight school districts in Terengganu were selected randomly where Marang and Hulu Terengganu were chosen. Thirteen schools met the inclusion criteria of which were 12 schools in rural Marang districts. Meanwhile, in Hulu Terengganu districts, there were 141 schools that met the inclusion criteria, of which 11 were in rural area; 2) Stage 2: One rural school was chosen randomly from each of the selected districts. The total class in Form II and IV for each schools were identified; 3) Stage 3: Three classes were selected randomly or each form: Form II and Form IV in each selected schools. Hence, total of 12 classes; 4) Stage 4: The students were identified randomly from the enrollment of each selected classes.

Data collection

Data were collected at schools in Hulu Terengganu and Marang, Malaysia. To ensure the quality of the data collected, the selected respondents were grouped into 17 groups, with 10 students in each group. Students were guided by the researcher to fill in the questionnaires. The weight and height of the respondents were measured and respondents were asked to fill the anthropometric data and socio-demographic information first followed by the KAP questionnaire and next, the food frequency questionnaire.

Research instruments

Other than anthropometric and socio-demographic information, respondents answered the KAP Questionnaire and Food Frequency Questionnaire (FFQ). These questionnaires have been tested for reliability and validity (Makumbe 2014; Daud *et al.* 2018; Harrington 2016; Mohamed *et al.* 2018). Pilot test was done prior to the study to test for the internal consistency. The internal consistency of the questionnaire was sufficient, with 0.720, 0.867 and 0.716 for knowledge, attitude and practices respectively. The total scores for each domain were calculated and categorized as poor (≤50% of total score), moderate (51% to 69%) and good (≥70% of total score) (Harrington 2016).

Knowledge domain. This domain consisted of nine closed-ended positive statement

questions regarding the general knowledge, recommendations and benefits of fruit intake. Each question was provided with three scales, 'yes', 'no' and 'not sure' answer with the marks of 2, 1 and 0 respectively. It gave a total score range of 0–18 for knowledge section.

Attitude domain. It consisted of 12 statements with five Likert scale answers provided. Likert scales answers were scored as follows: Strongly agree – 4; Agree – 3; Disagree – 2; Strongly disagree – 1; Not sure – 0, with the total score range of 0–48. 'Strongly agree' indicated maximum adherence towards fruit consumption. The statement was overall related to their belief towards benefits of fruits consumption.

Practice domain. It consists of eight questions with multiple choice answers as follows: 'always', 'sometimes', 'rarely' and 'never'. Subjects were asked regarding their practice towards fruit consumption, such as their habits of taking fruits in school and at home, fruits as a choice of snacking and the frequency of taking fruits. Each question regarding good practice was provided with a score of "4" for always, while the lowest practice was given "1" for never.

Food frequency questionnaire (FFQ). Respondents were asked specifically on the frequency of fruits consumption over the past 12 months (Mohamed *et al.* 2018). The frequency of fruit consumption was referred to the past 12 months in order to avoid the seasonal factors or narrow ranges of fruit intake among the adolescents. There were 15 types of seasonal fruit that were included in the FFQ which was chosen from the majority of the fruits that were among the respondents in the pilot study. The general format of the questionnaire contained instructions on how to use the FFQ, portion size options and frequency options with the help of visual illustrations. Frequency of fruit consumption was recorded in nine categories: 1) None per month; 2) One to three times per month; 3) One time a week; 4) Two to four times per week; 5) Five to six times per week; 6) One time a day; 7) Two to three times per day; 8) Four to five times per day and; 9) Six and more times per day) (Mohamed *et al.* 2018). In each category, there were options for how frequently each fruit item was consumed and the serving size of the fruit intake daily. The frequency of fruit intake was taken as an average, multiplied with the serving size of the fruit in a meal and categorized into groups of less than two servings per day, adequate intake of two serving per day, and more than two serving per day.

Data analysis

Data was entered and analyzed using IBM SPSS software version 22.0. Descriptive statistics was used to calculate and categorized the socio-demographic characteristics of subject, prevalence of fruit consumption, level of knowledge, attitude and practice of fruit consumption. Numerical data was presented as Median (IQR) since they were not normally distributed, while categorical data was presented as frequency and percentage. The score of knowledge, attitude and practices of fruit consumption of both genders were analyzed by using Mann-Whitney Test. The correlation among KAP variables was tested by using Spearman's Correlation Test. All tests were two-sided and a $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

This study involved 184 respondents, where 94 of them were female and the rest were male. The majority of female respondents were 16 years old (56.4%) while for the average age for male respondents was 14 years old (52.2%).

Anthropometric and socio-demographic information

The height and weight of all respondents were measured and BMI value were calculated based on the measured height and weight. The results indicated that majority of the respondents had normal weight and they were within normal BMI. They also had low-to-normal socio-economy status based on the location of their houses, number of siblings and current parents' occupation (Table 1).

Majority of the respondents came from Malay ethnicity and had larger household, consisting of six to ten members per family . More than half of their parents were self-employed, such as managing a small business and others. However, one third of the parents' respondents worked at the office.

Prevalence of fruit consumption

Fruit consumption among adolescents from school in both district, Hulu Terengganu

Table 1. Socio-demographic characteristics of respondents

Characteristics	Female (n=94) n (%)	Male (n=90) n (%)	Total (n=184)
Body mass index (kg/m^2)			
Severely thin	3 (3.2)	8 (8.9)	11 (5.9)
Thin	12 (12.8)	8 (8.9)	20 (10.9)
Normal	44 (46.8)	52 (57.8)	96 (52)
Overweight	15 (16.0)	11 (12.2)	26 (14)
Obese	20 (21.3)	11 (12.2)	31 (17.2)
Weight (kg)	51.496±13.61	52.765±17.50	
Height (cm)	149.921±6.26	157.846±8.50	
Body mass index (z-score)	0.554±1.46	0.175±1.60	
Socio-economy status			
High	15 (16)	10 (11)	25 (14)
Normal	37 (39)	35 (39)	72 (39)
Low	42 (45)	45 (50)	87 (47)
Parents occupation			
Employed	17 (18)	28 (31)	45 (24)
Self employed	52 (55)	40 (44)	92 (50)
Not employed	25 (27)	22 (25)	47 (26)
Number of household member			
3−5	20(21)	15 (17)	35 (22)
6−8	48 (51)	40 (44)	88 (45)
9 and above	26 (28)	35 (39)	61 (33)

and Marang was categorized into three groups which are group of less than two serving per day, group of adequate two serving and group of more than two serving (Malaysian Dietary Guidelines 2017) (Table 2).

Most of the respondents had inadequate fruit consumption (64.1%), followed by excessive fruit consumption (21.7%) and the last was adequate fruit consumption of 2 servings per day (14.1%). Only 13.8% and 14.4% of female and male respondents had adequate fruit consumption, respectively. Based on the data from food frequency and serving size, most of the student prefered local fruits such as orange, apple and bananas, due to the higher accessibility in rural areas.

Despite the study involved rural students, our study found that 61.1% of male students

Table 2. Percentage of fruit consumption among female and male adolescents in Hulu Terengganu and Marang districts in rural Terengganu (n=184)

Gender	<2 servings/day (95% CI)	2 servings/day (95% CI)	>2 serving/day (95% CI)
Overall (n %)	118 (64.1)	26 (14.1)	40 (21.7)
Female	63 (67.0)	13 (13.8)	18 (19.1)
Male	55 (61.1)	13 (14.4)	22 (24.4)

*Descriptive statistics test

and 67.0% female students had inadequate fruit consumption. This is in contradiction with the National Health & Morbidity Survey 2017 (NHMS), which reported higher prevalence of adequate fruit servings per day among Malaysian adolescents in rural areas and female respondent contributed the most (IPH 2017). However, another study proposed that fruit prevalence was higher among men because they believed that fruit was a high energy-dense food, which gave masculinity and power to them (Ward 2012). While on the other hand, female had higher probability and willingness to change their lifestyle and meet the fruit recommended intake serving due to dieting and good body image (Voelker *et al.* 2015).

Another determinant for fruit consumption is higher SES (Socioeconomic Status), since it allows greater chances to buy healthy food including fruits (Lallukka *et al.* 2010). However, this current study does not report on the SES relationship with the fruit consumption among respondents.

Respondents' knowledge, attitude and practices towards fruit consumption

Table 3 below showed that majority of female and male respondents had high knowledge level towards fruit consumption and no significant difference was found between male and female respondents with the percentage of 62.8% and 60.0% with the Median (IQR) of 14.00 (3.00) and 13.00 (3.00) respectively.

Only around a quarter, 24.5% and 26.7% of female and male, respondents who agreed with the statement of 'Consumption of fruits based on food pyramid is two servings respectively. This showed that the respondents knew about the benefit of fruit but not for its recommended amount.

Even though the results indicated high knowledge level among respondents, many did not know the recommended amount of fruit to be taken. This is similar to a study in Sarawak which found that two third of students were not aware of the recommended daily serving of fruit consumption (Aung *et al.* 2012). A slightly

Table 3. Level of knowledge towards fruit consumption among respondents (n=184)

Level of knowledge	Female n=94 n (%)	Male n=90 n (%)	p*
Poor (0−50)	4 (4.3)	4 (4.4)	
Moderate (51−69)	31 (33.0)	32 (35.6)	0.797
Good (70−100)	59 (62.8)	54 (60.0)	
Median (IQR)	14.00 (3.00)	13.00 (3.00)	

*Mann-whitney test

different level of knowledge between gender might be due to the fact that minority of male respondents might not be interested in nutritional knowledge (Kołłajtis-dołowy & Żamojcin 2016). Family education level, self-efficacy of the individua and environmental supports are some predictors that influenced the awareness and knowledge level regarding benefits of fruits among the respondent (Ismail *et al*. 2013).

In terms of attitude towards fruit consumption, the majority of respondents had good attitude towards food consumption with 63.8% of female respondents and 60.0% of male respondent had good attitude. The Median (IQR) were; 35.00 (6.25) for female respondents and 35.00 (6.00) for male respondents. There was no significant difference in terms of attitude score found between male and female (Table 4).

The highest percentage was found for the female respondents who strongly agreed with the statement of 'Eating more fruit will make me healthier' (62.8%); meanwhile, male respondents agreed more with the statement 'Fruits look appealing and appetizing' as compared to other statement in attitude domain. This showed different attitude approach in male and female respondents regarding fruit.

One of the reasons that rural adolescents has higher attitud toward fruit consumption was their believe on the beneficial effect of fruit to their body (Daud *et al.* 2018). The difference of self-efficacy of the respondent itself play a main role to influence the interconnection between self-efficacy towards healthy food and good dietary habit (Winzenberg *et al.* 2005).

More than half of the female respondents responded strongly agree for the statement 'fruits taste good to me'. The adolescents reported that the main reasons for lower fruit consumption among them were expensive price, low accessibility and low taste preferences towards fruit. However, the stronger predictor was knowledge regarding beneficial effects of fruit (Ilesanmi 2014).

Parental influence was another important determinant in influencing fruit consumption practice among adolescents. A study in Subang Jaya explained that most of the respondent completely relied on their parents in choosing and eating certain fruits (Ismail *et al.* 2013).

Even though majority of respondents showed good knowledge and attitude levels but they had moderate practice level towards fruit consumption. As shown in Table 5 below, most of the female and male respondents had moderate practice level followed by poor practice level and good practice level noted the least. The Median (IQR) for practice scores among female and male respondent were 17.00 (5.00) and 19.00 (4.25), respectively, with a statistically significant difference between both genders.

For the question of 'How often do you have fruit as a snack or as part of your meals?', only 12.8% of female and 11.1% of male respondents who reported regular intake of fruits, which showed poor practice level of fruit intake.

For the practice aspect, majority of the respondents had a moderate practice level towards fruit consumption, where male respondents who had a moderate practice level was slightly higher (58.9%) as compared to the female respondents (54.3%). The moderate practice level might be related to the availability of fruit at home or school. Low SES among rural area population might affect accessibility to fruit in terms of

Table 4. Attitude level towards fruit consumption among respondents (n=184)

Level of attitude	Female n=94 n (%)	Male n=90 n (%)	p*
Poor (0−50)	1 (1.1)	1 (1.1)	
Moderate (51−69)	33 (35.1)	35 (38.9)	0.404
Good (70−100)	60 (63.8)	54 (60.0)	
Median (IQR)	35.00 (6.25)	35.00 (6.00)	

*Mann-whitney test

Table 5. Practice level towards fruit consumption among respondents (n=184)

Level of practise	Female n=94 n (%)	Male n=90 n (%)	p*
Poor (0−50)	37 (39.4)	22 (24.4)	0.003
Moderate (51−69)	51 (54.3)	53 (58.9)	
Good (70−100)	6 (6.4)	15 (16.7)	
Median (IQR)	17.00 (5.00)	19.00 (4.25)	

*Mann-whitney test

money and travel distance to the supermarkets, except for the minority who have their own fruit farms (Dean 2011). Furthermore, previous study reported that dietary habits of male respondents was not influenced by the accessibility of healthy food at home, but the main factor contributed to it was their low taste preference towards healthy food including fruits.

Correlation between knowledge (K), attitude (A) and practice (P) score of fruit intake among the respondents

Table 6 below shows that knowledge and attitude score towards fruit intake had a statistically significant (p=0.036) but weak and positive correlation (r=0.155). However, there was no correlation reported between knowledge and practice scores. Meanwhile, weak, positive and statistically significant correlation can be seen between attitude and practice scores (p=0.007).

Current study also showed that there was statistically significant but weak and positive correlation between knowledge and attitude ($p<0.05$) and between attitude and practice ($p<0.001$), while no correlation was reported between knowledge and practice of fruit consumption. This indicates that the attitude towards fruit consumption among the respondents had lower likelihood to influence the knowledge and practice of fruit consumption among the respondents. Basically, knowledge, attitude and practice of the individual itself is not directly linked to each other, since it also can be influenced by other external factors such as socio-economic status or family lifestyle.

The level of correlation differed from the current study due to the respondents' bias, but both indicated positive correlation. A study in China also reported the similarly where they showed good attitude towards eating healthy, including fruit consumption, had a positive relationship with the dietary practice of the respondents (Yu *et al.* 2014).

Knowledge level has showed no relationship with the attitude and practice of fruit consumption among the respondents in this study, which was

Table 6. Correlation between knowledge, attitude and practice of fruit intake among respondents in rural Terengganu (n=184)

KAP domains	Total number of respondents (n=184 students)	
	r	p
Knowledge - attitude	0.155*	0.036
Knowledge - practice	-0.063	0.397
Attitude - practice	0.198**	0.007

*Spearman's correlation test

KAP: Knowledge, Attitude, Practise

in line with the study conducted by Banwat *et al.* (2012) where it reported that good knowledge was not strongly associated with the individual practice level. Outcome from this study found that out of 92.4% of their respondents who had moderate knowledge, 69.2% of them practiced fruit consumption daily, which shows that high level of knowledge was not strongly associated with fruit intake (Banwat *et al.* 2012). However, a study by Perera & Madhujith (2012) argued that knowledge was positively correlated to the individual practice of fruit consumption, where there was only 2.1% chances of an individual to increase fruit and vegetables consumption with better knowledge.

CONCLUSION

Majority of the adolescents recruited as respondent from Hulu Terengganu and Marang districts had good knowledge, good attitude and moderate practice towards fruit consumption. The knowledge, attitude and practice level towards fruit consumption among respondents can be considered acceptable, but it was not reflected on their fruit consumption since more than half of the respondents had inadequate fruit consumption. This might be associated with their limited knowledge on adequate amount of daily intake of fruit, in comparison to the overall knowledge about fruit consumption. Lastly, there was poor statistically significant positive correlation reported between knowledge and attitude ($p<0.05$) and between attitude and practice ($p<0.001$). Meanwhile, no correlation was reported between knowledge and practice of fruit consumption.

In future studies, it is encouraged to conduct study among respondents from both rural and urban areas in Terengganu and to involve samples that are representative of Malaysian adolescents population in rural areas. Future research requires further investigations of other factors influencing fruit consumption among growing adolescents during these critical periods and carried out by one-on-one interview by the researcher to reduce bias.

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DECLARATION OF INTERESTS

There is no conflict of interest involved in this study.

REFERENCES

Aung PP, Foung CS, Azman KB, Ain N, Zulkifeli NAB. 2012. Knowledge, attitude, and practice of healthy eating among the 1st and 2nd year students of Universiti Malaysia Sarawak (UNIMAS). 2012 International Conference on Nutrition and Food Sciences IPCBEE 39(2012):188−194.

Banwat ME, Lar LA, Daboer J, Audu S, Lassa S. 2012. Knowledge and intake of fruit and vegetables consumption among adults in an urban community in North Central Nigeria. The Nigerian Health Journal 12(1):12−15.

Boeing H, Bechthold A, Bub A, Ellinger S, Haller D, Kroke A, Leschik-Bonnet E, Müller MJ, Oberritter H, Schulze M *et al.* 2012. Critical review: Vegetables and fruit in the prevention of chronic diseases. Eur J Nutr 51(6):637−663. https://doi.org/10.1007/s00394−012-0380-y

Craigie AM, Lake AA, Kelly SA, Adamson AJ, Mathers JC. 2011. Tracking of obesity-related behaviours from childhood to adulthood: A systematic review. Maturitas 70(3):266−284. https://doi.org/10.1016/j.maturitas.2011.08.005

Daud NM, Fadzil NI, Yan LK, Makbul IAA, Yahya NFS, Teh AH, Rahman HA. 2018. Knowledge, attitude and practice regarding dietary fibre intake among Malaysian rural and urban adolescents. Malays J Nutr 24(1):77−88.

Dean WR, Sharkey JR. 2011. Food insecurity, social capital and perceived personal disparity in a predominantly rural region of Texas: An individual-level analysis. Soc Sci Med 72(9):1454−1462. https://doi.org/10.1016/j.socscimed.2011.03.015

Ham E, Kim HJ. 2014. Evaluation of fruit intake and its relation to body mass index of adolescents. Clin Nutr Res 3(2):126−133. https://doi.org/10.7762/cnr.2014.3.2.126

Harrington EM. 2016. Fruit and vegetable intake, attitudes, and beliefs of multicultural middle school students in Central Massachusetts. [Thesis]. Massachusetts: University of Massachusetts Amherts.

Hassan MR, Ghazi HF, Umar NS, Masri N, Jamil SM, Isa ZM, Safian, N. 2015. Knowledge, attitude and practice of healthy eating and associated factors among university students in Selangor, Malaysia. Pak J Nutr 14(12):892. https://doi.org/10.3923/pjn.2015.892.897

[IPH] Institute for Public Health. 2013. NHMS 2012-Malaysia School Based Nutrition Survey. Kuala Lumpur (KL): Ministry of Health Malaysia.

[IPH] Institute for Public Health. 2017. National Health and Morbidity Survey 2017: Adolescent Health Survey. Kuala Lumpur (KL): Public Health, National Institutes of Health, Ministry of Health Malaysia.

Ilesanmi O, Ilesanmi FF, Ijarotimi, IT. 2014. Determinants of fruit consumption among in-school adolescents In Ibadan, South West Nigeria. European Journal of Food Research & Review 4(2):100−109. https://doi.org/10.9734/EJNFS/2014/5087

Ismail N, Ab Karim MS, Karim R, Adzahan NM, Abd Halim N. 2013. Fruit and vegetable consumption behaviour: A qualitative study of Malay adults in Subang Jaya, Selangor. Journal of Agribusiness Marketing 6 (2013):52−67.

Kołłajtis-dołowy A, Żamojcin K. 2016. The level of knowledge on nutrition and its relation to health among Polish young men. Rocz Panstw Zakl Hig 67(2):155−161.

Lallukka T, Pitkäniemi J, Rahkonen O, Roos E, Laaksonen M, Lahelma E. 2010. The association of income with fresh fruit and vegetable consumption at different levels of education. Eur J Clin Nutr 64(3):324−327. https://doi.org/10.1038/ejcn.2009.155

Liu RH. 2013. Health-promoting components of fruits and vegetables in the diet. Adv Nutr 4(3):384S−392S. https://doi.org/10.3945/an.112.003517

Malaysian Dietary Guidelines. 2017. National Health and Morbidity Survey 2017: Adolescent Health Survey 2017. Kuala Lumpur (KL): Public Health, National Institutes of Health, Ministry of Health Malaysia.

Makumbe B. 2014. Knowledge, attitude, practices and factors affecting fruit and vegetable consumption in adolescents (Gokwe South District) [Dissertation]. Gweru: Midlands State University.

Menezes MC, Costa BVL, Oliveira CDL, Lopes ACS. 2017. Local food environment and fruit and vegetable consumption: an ecological study. Prev Med Rep 5:13−20. https://doi.org/10.1016/j.pmedr.2016.10.015

Mohamed K, Tin TS, Jalaludin MY, Al-Sadat N, Majid HA. 2018. Comparative validity of a food frequency questionnaire (MyUM Adolescent FFQ) to estimate the habitual dietary intake of adolescents In Malaysia. Asia Pac J Clin Nutr 27(4):898−907.

Perera T, Madhujith T. 2012. The pattern of consumption of fruits and vegetables by undergraduate students: A case study. Trop Agric Res 23(3):261. https://doi.org/10.4038/tar.v23i3.4663

Poh, BK, Ng BK, Siti Haslinda MD, Nik Shanita S, Wong JE, Budin SB, Ng LO, Khouw I, Norimah, AK. 2013. Nutritional status and dietary intakes of children aged 6 months to 12 years: Findings of the nutrition survey of Malaysian children (Seanuts Malaysia). Br J Nutr 110(S3):S21−35. https://doi.org/10.1017/S0007114513002092

Rojroongwasinkul N, Kijboonchoo K, Wimonpeerapattana W, Purttiponthanee S, Yamborisut U, Boonpraderm A, Kunapan P, Thasanasuwan W, Khouw I. 2013. Seanuts: The nutritional status and dietary intakes of 0.5−12-year-old Thai children. Br J Nutr 110(S3):36−44. https://doi.org/10.1017/S0007114513002110

Voelker DK, Reel JJ, Greenleaf C. 2015. Weight status and body image perceptions in adolescents: Current perspectives. Adolesc Health Med Ther 6:149−158. https://doi.org/10.2147/AHMT.S68344

Ward ML. 2012. Gender differences in fruit and vegetable consumption and Percent calories from fat among Latino Immigrants in North Carolina [Dissertation]. California: San Diego State University.

Winzenberg TM, Riley M, Frendin S, Oldenburg B, Jones G. 2005. Sociodemographic factors associated with calcium intake in premenopausal women: A cross-sectional

study. Eur J Clin Nutr 59(3): 463−466. https://doi.org/10.1038/sj.ejcn.1602105

[WHO] World Health Organization. 2014. Global strategy on diet, physical activity and health. https://www.who.int/publications/i/item/9241592222 [Accessed 15th October 2018].

[WHO] World Health Organization 2003. Diet, nutrition and the prevention of chronic diseases: Report of a joint WHO/FAO expert consultation (Vol 916). Diamond Pocket Books. http://apps.who.int/iris/bitstream/handle/10665/42665/WHO_TRS_916.pdf;jsessionid=EAE11A4E7357A2F69D34BDAC115802EE?sequence=1 [Accessed 15th October 2018].

Yu D, Zhang X, Gao YT, Li H, Yang G, Huang J, Zheng W, Xiang YB, Shu XO. 2014. Fruit and vegetable intake and risk of CHD: Results from prospective cohort studies of Chinese adults in Shanghai. Br J Nutr 111(2):353−362. https://doi.org/10.1017/S0007114513002328

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Characteristics of Yogurt Ice Cream Fortified with Red Dragon Fruit Puree as Anti-Obesity Functional Food

Siti Susanti*, Anang M. Legowo, Sri Mulyani, Yoga Pratama
Food Technology Study Program, Faculty of Animal and Agricultural Sciences,
Universitas Diponegoro, Semarang 50275, Indonesia

ABSTRACT

This study aimed to determine the effect of Red Dragon Fruit Puree (RDP) added to yogurt ice cream on its sensory, physical and chemical characteristics as well as its anti-obesity effect on mice fed with high-fat diet. To obtain the optimal addition level, RDP was added to the yogurt-based ice cream dough in various proportions: 0 (control), 10, 20, 30, and 40%. The sensory evaluation signifies that 30% RDP addition results in the best yogurt ice cream because it produces favorable flavor, attractive color (purplish pink), and the most preferable taste for the panelist. Data on physical and chemical characteristics showed that RDP yogurt ice cream has lower overrun, total solid, fat, carbohydrate, and calorie content than non-RDP yogurt ice cream ($p<0.05$). The animal test identified that consumption of RDP yogurt ice cream for 14 days could reduce the mice's (a xenograft model) body weight under normal and high-fat diet conditions ($p<0.05$). Thus, RDP yogurt ice cream with specific physical and chemical characteristics potentially results in anti-obesity effects in pre-clinic setting. RDP yogurt ice cream merits further studies, especially for clinical test, before the commercialization stage.

Keywords: antiobesity, ice cream, red dragon fruit, yogurt

INTRODUCTION

Ice cream is a dairy product greatly demanded by all walks of life. However, ice cream on the market contains high fat and sugar; therefore, people with obesity or on a diet program should avoid consuming ice cream (Su'i *et al.* 2020). Nowadays, yogurt ice cream has become a trend because in addition to its taste, yogurt ice cream also offers health benefits. Yogurt is a fermented dairy product using Lactic Acid Bacteria (LAB), namely, *Lactobacillus bulgaricus* and S*treptococcus thermophillus* (Aktar 2022). Yogurt is rich in protein, vitamins, minerals, and good bacteria beneficial for health. In contrast to other dairies, people with lactose intolerant can also consume yogurt because the fermentation process changes lactose to lactic acid, making it suitable for them (Yanni *et al.* 2020). Previous studies have stated that yogurt efficaciously reduces the risk of several significant chronic diseases, such as heart disease, diabetes, and cancer, and is healthy for consumption for obese people (Baspinar & Güldaş 2021). However, yogurt has a plain taste, less attractive appearance, and overpowering aroma of milk. Thus, some people do not like yogurt's plain taste.

Yogurt commonly consumed by adding pieces of fruit to improve the taste and add health benefits on the gut's health (Fernandez & Marrete 2017). Dragon fruit is a fruit with delicious taste and an attractive color. Besides its attractive appearance and sweet taste, dragon fruit has a high fiber content. Dragon fruit contains betacyanin and many other bioactive compounds, such as vitamins C, B, and E, flavonoids, carotenoids, and polyphenols, which are potential antioxidants (Fatmawati *et al.* 2018). Another advantage of red dragon fruit is rich in fiber, which is beneficial for people with obesity and diabetes. One hundred grams of dragon fruit contains 10.1 g of fiber, which can reduce fat absorption in the body (Fadlilah *et al.* 2021). Song *et al.* (2016) found that the betacyanin compound in dragon fruit significantly reduced weight gain in rats fed with a high-fat diet and 200 mg/kg of red dragon fruit for 14 days. However, there has been no research applying dragon fruit to food products, such as yogurt, which can function as

*Corresponding Author: tel: +6282171458795, email: sitisusanti@live.undip.ac.id
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an anti-obesity food. Therefore, this study aimed to determine the effect of adding Red Dragon Fruit Puree (RDP) with different concentrations on physical characteristics, nutrient content, and sensory characteristics of the yogurt ice cream. Further, this study also explored the effect of RDP in yogurt, as a functional food, on body weight of mice as the obesity test animal model

METHODS

Design, location, and time

The study consisted of four stages. The first was the organoleptic test, followed with physical and chemical characteristics tests and the final stage was in vivo test using mice models. The in vivo test for the anti-obesity trial employed a Completely Randomized Design (CRD). This study employed five treatments (T0: control, T1-4: 10, 20, 30, and 40% addition of red dragon fruit) and four replications. The best treatments according to the organoleptic test then was tested on mice for the in vivo test. The mice models were assigned randomly into the two groups: treatment group and control group.

This research was carried out at the Food Chemistry and Nutrition Laboratory, Faculty of Animal Husbandry and Agriculture, Universitas Diponegoro in September-December 2019. The study protocol was approved by the Faculty of Medicine, Universitas Diponegoro, using a method based on the Ethical Clearance of No.127/EC/H/KEPK/FK-UNDIP/X//2019.

Materials and Tools

The main ingredients of this study were cow's milk, yogurt starter (*biokul*), red dragon fruit, sugar, and skimmed milk. The tools used in this research were a container, filter cloth, blender, analytical balance (DJ Excellent Scale, USA), stopwatch, oven, desiccator, Bunsen, porcelain cup.

Procedures

Preparation of red dragon fruit puree (RDP)

Red dragon fruit puree was made by cutting the red dragon fruit into four parts and taking the flesh by separating it from the skin and chopping it into pieces. The red dragon fruit was weighed according to the treatment and was then blended until became pulp. Then, it was filtered using a filter cloth. The formulations of RDP yogurt ice cream were 500 ml of milk, 15 g of yogurt starter, 25 g of sugar, 30 ml of skim milk, and red dragon with each treatment of T0-4: 0; 50; 100; 150; and 200 g.

Preparation of red dragon fruit yogurt ice cream (RDYIC)

To make the yogurt, 500 ml fresh cow's milk was pasteurized for 15 minutes at 72°C. Then, it was put into a sterilized container or jar. Afterward, the dragon fruit puree was mixed according to the treatment T1-4: 10, 20, 30, and 40% of the addition of red dragon fruit from the total milk used (v/v). The mixture was then stirred until homogeneous. The bacterial starter was weighed for 3% of 500 ml of milk and then inoculated into a jar containing pasteurized milk and fresh dragon fruit puree. The jars were tightly closed and incubated for 12 h at 43°C. The next step was adding sugar and skim milk. Then, the mixture was stirred well, mixed with the yogurt mixture, and finally pasteurized again for 30 minutes at a temperature of 85°C. The produced yogurt ice cream was then put into a freezer at -18°C for approximately 15 h prior to further analysis.

Sensory test

Organoleptic testing was carried out using an objective sensory measurement with the attribute rating test of taste, color, texture, and aroma; in addition it also included the overall acceptance. The assessment was carried out using 25 semi-trained panelists. The taste attribute employed five levels: 1 (very sour), 2 (sour), 3 (quite sour), 4 (slightly sour), and 5 (not sour). Yogurt aroma attributes used five levels: 1 (not fruity), 2 (slightly fruity), 3 (fairly fruty), 4 (fruity), and 5 (very fruity). The color attribute employed five levels: 1 (not purple), 2 (slightly purple), 3 (quite purple), 4 (purple), and 5 (strong purple). Texture attributes have five levels: 1 (not soft), 2 (slightly soft), 3 (quite soft), 4 (soft), and 5 (very soft). Meanwhile, the overall acceptance of the preference used a scale of 1 (dislike), 2 (like slightly), 3 (like moderately), 4 (like), and 5 (likes extremely). The best RDYIC formula was measured by the highest overall organoleptic score. The RDYIC which scored best in the sensory test was then tested for physical and chemical analysis as well as in-vivo test on mice.

Physical characteristics test

The physical characteristic test of ice cream included the overrun analysis (Goff & Hartel 2013) and the total solid analysis (Singo & Beswa 2019). The overrun measurement was done by measuring the ice cream dough with the same volume using a measuring cup before and after the process with an ice cream maker. Total solids analysis was conducted by calculating the moisture content. Afterward, the moisture content was used to calculate the total solids by deducting 100% with the percentage of moisture content.

Nutrient content analysis

The test for chemical characteristics of ice cream included proximate and calorie content tests. The proximate tests measured the moisture content and ash content following the AOAC method (Wong *et al.* 2019), the protein content based the Kjeldahl method (Chen *et al.* 2019), the fat content following the Soxhlet method, and the carbohydrate test by difference (Góral *et al.* 2018). The calorie content test employed the proximate test results by calculating the number of carbohydrates, fats, and proteins with 9 kcal of calories produced by 1 g of fat and 4 kcal calories produced by 1 g of protein or 1 g of carbohydrates (Balthazar *et al.* 2017).

Pre-clinical test of red dragon fruit yogurt ice cream as anti-obesity functional food

The pre-clinical test (in vivo) was done on mice as experimental animals and was conducted to determine the effect of RDPYIC with the best organoleptic test result on the mice's weight gain. The pre-clinical test has been approved by the Ethical Clearance No.127/EC/H/KEPK/FK-UNDIP/X//2019. The pre-clinical trial was conducted in 12 mice aged 2–3 months and weighed 20–35 g. These mice were obtained from mouse breeders in Banyumanik. Pre-clinical trials were conducted for five weeks using a force-feeding method. The variety of feed given was Normal Feed (ND) and High-Fat Feed (HFD). The ND group received commercial feed while the HFD group received a mixture of commercial feed and high-fat components: egg yolk and coconut oil. The provision of yogurt ice cream was adjusted to an acceptable dose or the Acceptable Daily Intake (ADI) converted according to the mice's weight with 0.728 g/weight of mice. The normal diet treatment referred to providing commercial feed to the mice while the high-fat diet referred to providing a mixture of commercial feed (80%), egg yolk (10%), and coconut oil (10%).

The pre-clinical test was conducted using the optimal treatment based on the organoleptic test, which was additon of 30% of red dragon fruit. The rest of the feed was weighed every day, and the body weight of the mice was measured at the beginning and end of the treatment.

Data analysis

The experimental method of this research was a Completely Randomized Design (CRD) with a single factor of five treatments and four replications. The physical and chemical characteristics data (overrun, total solids, proximate, and calorie content) were compared between the best score in RDYIC formula and regular yogurt without any RD addition using t-test. The organoleptic test data were analyzed using the Kruskal Wallis non-parametric test. If an effect of treatment on the organoleptic properties of yogurt ice cream had been found, a Mann-Whitney follow-up test was carried out. The data for pre-clinical trials were statistically analyzed using the t-test. All obtained data were analyzed using the SPSS 22.0 at a significant level of 0.05.

RESULTS AND DISCUSSION

Sensory of characteristics red dragon fruit puree yogurt ice cream

The results of the organoleptic test of red dragon fruit puree yogurt ice cream are presented in Table 1. The RDP addition affects the aroma, taste, color, texture, and the overall acceptance of yogurt ice cream. The dominant aroma and taste of yogurt in the treatment T0 (control) resulted from the main ingredient, namely fermented cow milk. Therefore, the yogurt has a characteristic taste and sour aroma that are produced during the milk fermentation process.

Table 1 shows that non-RDP yogurt higher rates in term texture and aroma compared to all RDP treatments and the difference was statistically significant. The 20% RDP had the highest rate in taste but it was not statically different compared to other RDP treatments. The 10% formula had the highest score in texture but it was not statistically different compared to other RD treatments. The 40% formula had the highest rate in color compared to other formulas and the

Table 1. Sensory characteristics of red dragon fruit puree yogurt ice cream

Attributes	Addition of red dragon puree (%)					Rating interpretation (1–5)
	0	10	20	30	40	
Aroma	4.32±0.46[a]	3.12±0.46[b]	2.82±0.51[b]	3.10±0.72[b]	2.31±0.15[b]	Not fruity–Fruity
Taste	2.12±0.82[a]	3.10±0.55[b]	3.11±0.51[b]	3.07±0.32[b]	3.09±0.53[b]	Very sour–Sour
Color	1.58±0.59[a]	2.76±0.23[b]	3.22±0.48[c]	3.72±0.41[d]	4.46±0.48[e]	Not purple–Strong purple
Texture	4.10±0.55[a]	3.29±0.44[b]	3.13±0.57[b]	3.54±0.69[b]	3.58±0.25[b]	Not soft–Very soft
Overall acceptance	3.51±0.47[abc]	3.22±0.65[c]	3.28±0.72[bc]	4.32±0.78[a]	3.82±0.69[ab]	Dislike–Like extremely

The data were expressed as mean±standard deviation
Different superscripts in the same row show a significant difference ($p<0.05$)

differences statistically significant. The more RDP added to the yogurt ice cream, the stronger the aroma and color. Thus, RDP affects the taste, aroma, color, and texture differently. Hence, the overall score for all 5 formulas showed that the T3 RDP treatments with 30% addition of RDP received the highest score and it was most preferred in terms of overall acceptance and it was used for further test.

The sweet taste and the distinctive aroma of red dragon fruit may disguise the aroma of yogurt. However, red dragon fruit has a strong unpleasant aroma; thus, the addition large ammount of dragon fruit juice could reduce the panelists' acceptance. Meanwhile, the taste of yogurt can be influenced by the addition of other ingredients, such as honey and low-calorie sweetener functioning as a sweetener. Red dragon fruit has a fairly sweet taste; therefore, this fruit is suitably made into a yogurt ice cream fortifier that can compensate the sour taste in yogurt. The sour taste in yogurt occurs during the fermentation process by LAB, which produces lactic acid, citric acid, and acetic acid; thus, the lower the pH of fermented milk, the sourer the yogurt taste (Andila & Pato 2018). In the control treatment, the resulted taste was too sour, which affected the level of panelists' acceptance.

Higher addition of red dragon fruit puree also significantly affected the samples' color because the addition of RDP produce stronger color of purple. The stronger color was produced by the increasing betacyanin pigment content in the ice cream (Thaiudom *et al.* 2021). The addition of red dragon fruit puree produce a slightly softer yogurt ice cream texture because red dragon fruit has a high moisture content, which can form ice crystals during freezing.

Physical characteristics of yogurt ice cream with red dragon fruit addition

Table 2 shows that the overrun and total solids values of RDP yogurt ice creambetween T0 and T3 formula were significantly different ($p<0.05$). The overrun value of yogurt ice cream with the addition of RDP decreased significantly than the control. The decrease in overrun value was caused by red dragon fruit juice, which had a fairly high fiber content. The high fiber content in fruits results in decreased overrun value because fiber can affect the viscosity of the ice cream mix, which binds air; thus, ice cream mix is difficult to expand (Hapdang *et al.* 2021). In addition, the fiber in red dragon fruit can bind water so that the ice cream mix got thick and as a result, the total solids of the ice cream added with RDP would also decrease. Therefore, more quantity of red dragon fruit puree added would add more moisture into the ice cream content and reduced the total solids. Masykuri *et al.* (2012) stated that the lower the total solids, the greater the amount of frozen water; thus as a result, less air could enter and cause ice cream overrun to be low.

Table 2. Physical characteristics of red dragon fruti puree yogurt ice cream

Parameters	Treatment	
	Yogurt ice cream	Red dragon fruit puree yogurt ice cream (30%)
Overrun (%)*	21.57±2.35	14.32±1.37
Total Solids (%)*	44.83±2.87	36.23±2.57

*Significant differences between yogurt ice cream versus red dragon pure yogurt ice cream ($p<0.05$)

Nutrient content of red dragon fruit puree yogurt ice cream

The results of the nutrient analysis are presented in Table 3. The results of the independent sample t-test show that the addition of red dragon fruit puree had a significant effect on moisture, fat, carbohydrate, and calorie content but there was no significant effect on ash and protein content. The differences in the moisture content of the two formulas were caused by the addition of red dragon fruit puree, which had3 a fairly high moisture content. According to Herianto *et al.* (2015), red dragon fruit flesh had 85.77% of moisture content. The moisture content in ice cream tended to increase when the addition of red dragon fruit puree was increased. The ash content in both treatments had a fairly low value. The protein content of yogurt ice cream increased along with the addition of red dragon fruit puree because dragon fruit contained 0.53% protein (Nguyen *et al.* 2021). This result is in line with Nurul and Asmah (2014), who found 100 g of red dragon fruit flesh contained 0.159–0.229 g of protein.

The significance decrease in fat content of red dragon fruit puree yogurt ice cream happened because the RDP concentration added in the yogurt was quite large, namely 30%. Red dragon fruit has a very low-fat content of 0.6 g per 100 g; thus, greater RDP addition in yogurt will reduce the fat content of yogurt (Zahro & Nisa 2014). In addition, during the fermentation process, fats undergo hydrolysis into simpler compounds by lipase that are produced by lactic acid bacteria (Triasih & Priyadi 2021). The decreasing carbohydrate levels in red dragon fruit puree yogurt ice cream were influenced by different methods in yogurt ice cream. Lactic acid bacteria convert carbohydrates in the sample into simple sugars that are used as an energy source; thus, carbohydrates in the form of natural sugars would decrease. Julianto (2016), stated that lactic acid bacteria in yogurt convert carbohydrates into monosaccharides, which will be used to produce lactic acid. The total calorie content of sample with 30% red dragon fruit puree decreased because T3 had lower fat and carbohydrate content than T0.

Anti-obesity effect of red dragon yogurt ice cream

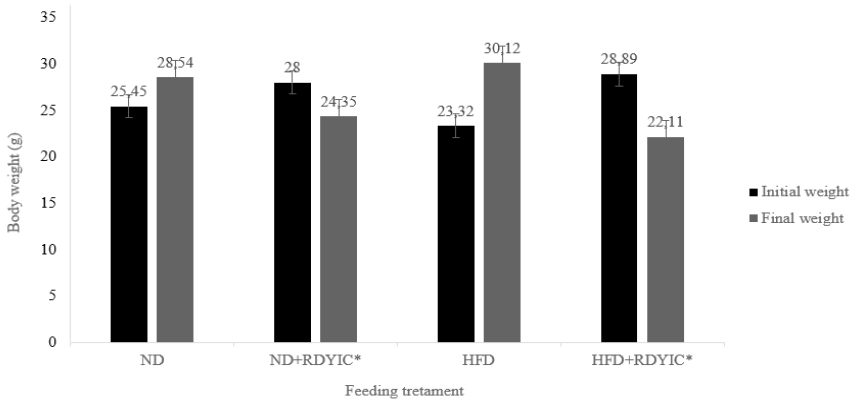
Preclinical test was carried out to examine the effect of RDP yogurt ice cream on changes of body weight of mice. The T3 or 30% addition of red dragon fruit puree into yogurt ice cream was given for the treatment group. All groups that were treated for 14 days showed a significant difference in initial weight and final weight, except for the mice treated with Normal Diet (ND) only. The administration of a normal diet for 14 days did not significantly change the weight of the mice, but the administration of RDP yogurt ice cream with ND significantly decreased the final weight of the mice ($p<0.05$). Meanwhile, the HFD treatment significantly increased the body weight of mice. On the other hand, feeding HFD combined with RDP yogurt ice cream showed significant reduction in the body weight of mice. Thus, the weight loss experienced by mice with ND and HFD diets could be associated with the consumption of RDP yogurt ice cream (Figure 1).

Adhi *et al.* (2018) have proven that fiber in red dragon fruit inhibits fat absorption and allows them to be eliminated with feces. Soluble fiber forms a thick solution that inhibits the digestion

Table 3. Chemical characteristics of red dragon fruit puree yogurt ice cream

Parameters	Treatment	
	Yogurt ice cream	Red dragon fruit puree yogurt ice cream (30%)
Moisture content (%)*	52.11±1.85	64.70±0.78
Ash Content (%)	0.72±0.24	0.67±0.02
Protein Content (%)	2.36±0.74	3.12±0.13
Fat Content (%)*	8.18±0.52	5.36±0.21
Carbohydrate content (%)*	34.88±2.73	22.11±0.74
Calorie content (kcal/g)*	222.58±8.44	149.16±4.51

*Significant difference between yogurt ice cream and red dragon fruit puree yogurt ice cream ($p<0.05$)



The mice were fed with a Normal Diet (ND); Normal diet+30% Red Dragon Fruit Puree Yogurt Ice Cream (ND+RDYIC); High-Fat Diet (HFD) and; High-Fat Diet+30% Red Dragon Fruit Puree Yogurt Ice Cream (HFD+RDYIC)
The data are expressed as a mean of ± standard deviation.
*Significant difference between the initial weight and final weight

Figure 1. Comparison of mice's body weight before and after the feeding treatment for fourteen days

and absorption of carbohydrates and fats. Otherwise, fiber can be fermented by intestinal flora into Short-Chain Fatty Acids (SCFA); one of them is butyrate, the main SCFA in the human intestine. SCFA production will affect appetite either directly in the brain or via different signals (Astuti & Jenie 2020; Hjorth *et al.* 2019). Consuming fiber prolong the period of fullness due to the expansion of the large intestine. Dietary fiber also promotes growth of beneficial microbes in the gut, particularly lactobacilli. Gut microbiota can influence bile acid metabolism and produce various metabolites, including SCFA and neuroactive. Meanwhile, negligible protein sequences can be translocated to the peripheral circulation or interact with enteroendocrine cells and systems, which causes release of neuropeptides and peripheral hormones related to appetite and eating behavior (Esmaeili *et al.* 2022). Consumption of fiber regularly can help to lose weight while daily intake of yogurt can help reduce visceral fat by 81% since the high protein content in yogurt helps the body to metabolize fat faster (Michels *et al.* 2020).

CONCLUSION

Addition of RDP to yogurt ice cream produced red dragon fruit puree yogurt ice cream (RDYIC) with more fruity aroma, less sour taste, more purple color, and softer texture. Yogurt with 30% RDP was the most preferred compared to other formulas. RDYIC had a high moisture content with low overrun and total solids values, fat content, carbohydrate content, and total calorie content. Preclinical (in vivo) test found that the body weight of mice decreased when they consumed the RDYIC for 14 days in addition to both normal diet and high fat diet. Thus, RDYIC has a potential as functional food to be used as dessert product with an anti-obesity property.

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DECLARATION OF INTERESTS

The authors have no conflict of interest.

REFERENCES

Adhi NGMAD, Suastuti NWB, Putra AAB. 2018. vActivity of Hylocereus Costarioensis's Extract as Antiobesity and hypolipidemic of obese Rats Int J Pharm Res Allied Sci 7(1):201–208.

Aktar T. 2022. Physicochemical and sensory characterisation of different yoghurt production methods. Int Dairy J

125:105245. https://doi.org/10.1016/j.idairyj.2021.105245

Andila R, Pato U. 2018. Pembuatan susu fermentasi probiotik menggunakan *Lactobacillus casei* subsp. Casei R-68 dengan penambahan ekstrak buah naga merah. J Sagu 17(2):37–44.

Anggraeni D, Adji D, Sutrisno B. 2012. Identifikasi leptin pada kesembuhan luka tikus yang diberi pakan lemak tinggi dan aplikasi zinc topikal. J Veteriner 13(4):395−401.

Astuti Y, Jenie IM. 2020. Correlation of body mass index on waist circumference and blood pressure. Int J Public Health 9(4):273–278. https://doi.org/10.11591/ijphs.v9i4.20443

Balthazar CF, Silva HLA, Cavalcanti RN, Esmerino EA, Cappato LP, Abud YKD, Moraes J, Andrade MM, Freitas MQ, Sant'Anna C *et al*. 2017. Prebiotics addition in sheep milk ice cream: A rheological, microstructural and sensory study. J Funct Foods 35:564–573. https://doi.org/10.1016/j.jff.2017.06.004

Baspinar B, Güldaş M. 2021. Traditional plain yogurt: a therapeutic food for metabolic syndrome. Crit Rev Food Sci Nutr 61(18):3129–3143. https://doi.org/10.1080/10408398.2020.1799931

Chen W, Liang G, Li X, He Z, Zeng M, Gao D, Chen J. 2019. Effects of soy proteins and hydrolysates on fat globule coalescence and meltdown properties of ice cream. Food Hydrocoll 94:279–286. https://doi.org/10.1016/j.foodhyd.2019.02.045

Esmaeili M, Ajami M, Barati M, Javanmardi F, Houshiarrad A, Mousavi KA. 2022. The significance and potential of functional food ingredients for control appetite and food intake. Food Sci Nutr 10(5):1602–1612. https://doi.org/10.1002/fsn3.2783

Fadlilah S, Sucipto A, Judha M, Amestiasih T, Nekada CDY, Mindarsih E, Pramana C. 2021. Red dragon fruit (Hylocereus Polyrhizus) to reduce cholesterol level in people with excessive nutritional status. Indian J Forensic Med Toxicol 15(4):2557–2565. https://doi.org/10.37506/ijfmt.v15i4.17090

Fatmawati F, Laenggeng AH, Amalinda F. 2018. Analisis kandungan gizi makro kerupuk buah naga merah (hylocereus polyrhizus). J Kolaboratif Sains 1(1). https://doi.org/10.56338/jks.v1i1.347

Fernandez MA, Marette A. 2017. Potential health benefits of combining yogurt and fruits based on their probiotic and prebiotic properties. Adv Nutr 8(1):155S–164S. https://doi.org/10.3945/an.115.011114

Goff HD, Hartel RW. 2013. Ice Cream: Ice Cream Structure. 7th Edition. New York (USA): Springer.

Góral M, Kozłowicz K, Pankiewicz U, Góral D, Kluza F, Wójtowicz A. 2018. Impact of stabilizers on the freezing process, and physicochemical and organoleptic properties of coconut milk-based ice cream. LWT 92:516–522. https://doi.org/10.1016/j.lwt.2018.03.010

Hapdang A., Maruddin F, Malaka R. 2021. Physical characteristics of ice cream with the addition of tangerines (Citrus reticulata). In IOP Conference Series: Earth and Environmental Science 788(1): 012105. IOP Publishing. https://doi.org/10.1088/1755-1315/788/1/012105

Herianto AF, Hamzah, Yusmarini. 2015. Studi pemanfaatan buah pisang mas (Musa acuminata) dan buah naga merah (Hylocereus polyrhizus) dalam pembuatan selai. JOM Fakultas Pertanian Universitas Riau 2(2):1–11. https://doi.org/10.7454/psr.v2i1.3333

Hjorth MF, Blædel T, Bendtsen LQ, Lorenzen JK, Holm JB, Kiilerich P, Astrup A. 2019. Prevotella-to-Bacteroides ratio predicts body weight and fat loss success on 24-week diets varying in macronutrient composition and dietary fiber: Results from a post-hoc analysis. Int J Obes 43(1):149–157. https://doi.org/10.1038/s41366-018-0093-2

Julianto B, Rossi E, Yusmarini Y. 2016. Karakteristik kimiawi dan mikrobiologi kefir susu sapi dengan penambahan susu kedelai. Jom Faperta 3(1).

Masykuri M, Pramono YB, Ardilia D. 2012. Resistensi pelelehan overrun dan tingkat kesukaan es krim vanilla yang terbuat dari bahan utama kombinasi krim susu dan santan kelapa. JATP 1(3):78−82.

Michels KB, Willett WC, Vaidya R, Zhang X, Giovannucci E. 2020. Yogurt consumption and colorectal cancer incidence and

mortality in the nurses' health study and the health professionals follow-up study. Am J Clin Nutr 112(6):1566–1575. https://doi.org/10.1093/ajcn/nqaa244

Nguyen TTH, Pham DC, Chu TP, Vu NH, Samhaber WM, Nguyen MT. 2021. Impact of jeva evaporation on storage stability and physiochemical characteristics of Vietnam red dragon fruit (hylocereus Polyrhizus). Chem Eng Trans 87:169–174.

Nurul SR, Asmah R. 2014. Variability in nutritional composition and phytochemical properties of red pitaya (Hylocereus polyrhizus) from Malaysia and Australia. Int Food Res J 21(4).

Singo TM, Beswa D. 2019. Effect of roselle extracts on the selected quality characteristics of ice cream. Int J Food Prop 22(1):42–53.https://doi.org/10.1080/10942912.2019.1567535

Song H, Chu Q, Yan F, Yang Y, Han W, Zheng X. 2016. Red pitaya betacyanins protects from diet-induced obesity, liver steatosis and insulin resistance in association with modulation of gut microbiota in mice. J Gastroenterol Hepatol 31(8):1462–1469. https://doi.org/10.1111/jgh.13278

Su'i M, Pradana HA, Anggraeni FD. 2020. Substitution of coconut milk and soybean tempeh flour to produce ice cream that qualified and healthy. N a J Adv Res 8(2):026–034. https://doi.org/10.30574/wjarr.2020.8.2.0400

Thaiudom S, Oonsivilai R, Thaiwong N. 2021. Production of colorant powder from dragon fruit (Hylocerecus polyrhizus) peel: Bioactivity, heavy metal contamination, antimutagenicity, and antioxidation aspects. J Food Process Preserv 45(1):1–26. https://doi.org/10.1111/jfpp.15044

Triasih D, Priyadi DA. 2021. Kajian tentang pengembangan eggurt dengan fortifkasi edamame sebagai agen antioksidan. J Peternak Indone 23(2):108–114. https://doi.org/10.25077/jpi.23.2.108-114.2021

Wong P, Ling HS, Chung KC, Yau TMS, Gindi SRA. 2019. Chemical Analysis on the Honey of Heterotrigona itama and Tetrigona binghami from Sarawak, Malaysia. Sains Malays 48(8):1635–1642. https://doi.org/10.17576/jsm-2019-4808-09

Yanni AE, Kartsioti K, Karathanos VT. 2020. The role of yoghurt consumption in the management of type II diabetes. Food Funct 11(12):10306–10316. https://doi.org/10.1039/D0FO02297G

Zahro C, Nisa FC. 2014. Pengaruh penambahan sari anggur (vitis vinifera l.) Dan penstabil terhadap karakteristik fisik, kimia dan organoleptik es krim [in press september 2015]. J Pangan dan Agroindustri 2014:3(4).

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Effect of Locally Produced Ready-to-Use Therapeutic Food on Children under Five Years with Severe Acute Malnutrition: A Systematic Review

Rimbawan Rimbawan[1*], Zuraidah Nasution[1], Puspo Edi Giriwono[2,3], Kharisma Tamimi[4], Khaerul Fadly[4], Astrid Noviana[4]

[1]Department of Community Nutrition, Faculty of Human Ecology, IPB University, 16680 Bogor, Indonesia
[2]Department of Food Science and Technology, Faculty of Agricultural Technology, IPB University, 16680 Bogor, Indonesia
[3]South-East Asia Food and Agricultural Science and Technology (SEAFAST) Center, IPB University, 16680 Bogor, Indonesia
[4]Postgraduate in Nutrition Science, Department of Community Nutrition, Faculty of Human Ecology, IPB University, 16680 Bogor, Indonesia

ABSTRACT

This review assessed the effect of Ready-to-Use Therapeutic Food (RUTF) on children under five years with Severe Acute Malnutrition (SAM). The reviewed studies were obtained from six databases. Using the search strategy, 3,521 studies were selected. After title and abstract screening, 75 studies were obtained for further full article screening. The inclusion criteria were types of study (RCT, quasi-RCT, or crossover), participants (SAM children aged 6−60 months with no complications), interventions (locally produced RUTF and standard RUTF), and outcome measures (recovery rate, mortality rate, weight gain rate, height gain rate, length of stay, weight-for-age z score, height-for-age z score, weight-for-length z score, anemia status, blood iron status, serum albumin, plasma amino acid level, adverse effects and acceptability of RUTF). A total of 33 studies were included in this review. Nine out of twenty-two studies that used standard RUTF had positive effects on recovery outcomes in children with SAM. The alternative RUTF produced from local protein sources showed slightly lower positive effects on SAM treatment than those of standard RUTF. Since the studies used different methods to assess the outcome, no formula could be selected as the best formula and selection should be made based on individual research objectives. In conclusion, both standard and the alternative locally produced RUTF can be applied for treatment of SAM by considering the local preferences, ingredients availability, production sustainability and product safety.

Keywords: CMAM, local protein sources, RUTF, SAM, weight gain

INTRODUCTION

The Basic Health Survey performed by the Indonesian Ministry of Health in 2018 showed that there had been an improvement in the nutritional status of children under five years in Indonesia (Ministry of Health of Republic of Indonesia (MoH RI) 2018). Stunting decreased from 37.2% in 2013 to 30.8% in 2018, while wasting decreased from 12.1% in 2013 to 10.2% in 2018. The wasting prevalence data indicate that severe wasting is still high, where 5.3% and 3.5% of children were severely wasted in 2013 and 2018, respectively. It means that almost one million children under five years of age in Indonesia experience severe acute malnutrition. Even though the number is decreasing, the decline was still deemed insignificant since the World Health Organization (WHO) set the limit of malnourishment prevalence to 20%. Moreover, the reduction of stunting is also one of the targets of Sustainable Development Goals (SDGs), which is in the second goal of "zero hunger." Therefore, along with the United Nations (UN), Indonesia is committed to eradicating any forms of malnourishment, including achieving the world's target regarding stunting and wasting in children by 2025.

Childhood malnutrition may bring long-term effects, i.e. cognitive impairment, delayed motor growth, poor physical performance, low birth weight of future offspring, behavioral

[*]**Corresponding Author:** tel: +62818705159, email: rimbawan@apps.ipb.ac.id
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issues, poor academic performance and low productivity in adulthood (Victoria *et al.* 2008). One of intervention methods that has been used in many countries is the provision of Ready-to-Use Therapeutic Food (RUTF) and Ready-to-Use Supplementary Food (RUSF). Children with severe and acute malnutrition that are managed as inpatients or outpatients can be given RUTF. Many studies have assessed the efficacy and effectiveness of various types of both RUTF and RUSF. Both are lipid-based products fortified with nutrients to treat acute malnutrition in children aged 6−59 months. Their formulations have to comply with WHO specifications. They have to be produced as products that require no cooking so that they can be immediately consumed by the targeted consumers. They are formulated to be high in energy and protein to support the recovery and weight gain of acutely malnourished children by rebuilding their lean tissues and supplementing their dietary nutrient deficiencies (De Pee & Bloem 2009).

Although they seem to be similar, RUSF and RUTF have some differences. RUSF is used to manage Moderately Acute Malnourished (MAM) children aged 6−59 months, while RUTF is used to treat Severely Acute Malnourished (SAM) children of the same age. RUTF can be provided for SAM children admitted in Outpatient Therapeutic Care (OTC) at local health centers with the condition that the children do not have any other medical complications and their appetite and sensory detection skills are not compromised. The dose of RUSF is standardized at 500 kcal/day, while the RUTF dose depends on the children's weight. When there is an improvement in weight gain, the dose should be readjusted. There has been a guideline available for the production of RUTF issued by the joint committee of WHO and FAO in 2019 (Codex Alimentarius Commission 2019).

Few reviews have been published on RUTF (Schoonees *et al.* 2019; Potani *et al.* 2021), however they did not specifically discuss the effectiveness of locally produced alternative RUTFs as compared to the standard RUTF. Therefore, this review was conducted to identify the effect of RUTF on children under five years old children with SAM by including studies that used locally produced alternative RUTFs. Several outcome measures were included as indicators namely anthropometric measurements, biochemical markers, adverse effects and the acceptability test. Findings from this review will provide insights for health practitioners or organizations involved in clinical guidelines development as well as policy makers dealing with SAM.

METHODS

Search strategy

Studies were searched from several databases (The Cochrane Central Register of Controlled Trials, PubMed, Science Direct, Wiley online library, BMJ global health and Oxford Academic). The search strategy did not apply language and date restrictions. The keywords used were "RUTF, SAM", "RUTF and SAM", "ready to use therapeutic food", "ready to use therapeutic food and severe acute malnutrition", "severe acute malnutrition" and "ready to use therapeutic food". The snowball technique was also used to search studies from references listed in related studies. A critical appraisal of the literature screened, selected and included for use in this study adhered to the guidelines as recommended by Young & Solomon (2009).

Studies were selected based on predefined inclusion criteria including types of study (Randomized Controlled Trial (RCT), quasi-RCT or crossover), participants (SAM children aged 6−60 months with no medical complication; weigh-for-height z score under -3 standard deviation; middle upper arm circumference under 115 mm; oedema exist), interventions, and outcome measures (recovery rate, mortality rate, weight gain rate, height gain rate, middle upper arm circumference gain length of stay, weight-for-age z score, height-for-age z score, weight-for-length z score, anemia status, blood iron status, serum albumin, plasma amino acid level, adverse effects and acceptability of RUTF). The exclusion criteria were record duplication, intervention strategy without RUTF, type of study using observational review, subject without SAM, subjects' age other than 6−60 months and outcome measures other than stated in the inclusion criteria.

Data collection and extraction

All records were screened by titles and abstracts obtained from the search and studies that met the predetermined eligibility criteria

were selected. In manuscript screening, at least two out of six authors agreed to select a manuscript. When there was a disagreement, then another author would be asked for opinion. The results of the screening steps were presented in Table 1. Once a manuscript was selected, the full text was accessed and information on each of the following aspects: study design, intervention (subject group, dose and duration), outcome and results, were extracted. Result synthesis was made based on the strength of the evidence obtained from the selected studies.

RESULTS AND DISCUSSION

The search strategy performed in this review yielded 3,521 records. From those records, as many as 3,220 studies were excluded based on title and abstract screening, leaving about 301 studies. Next, as many as 226 studies were excluded because they did not include RUTF in their treatment for children with SAM. After that, 43 studies were excluded since they did not fulfill the inclusion criteria. Finally, a total of 33 studies were included in this review. The outcomes observed was taken from a total of 18,668 subjects from all the studies included (Table 1). This shows the strength of the evidence of this review. Figure 1 shows the flow of the systematic review and Table 1 shows the details of the 33 studies.

Recovery rate

Thirteen studies evaluated the effect of RUTF on recovery of children with SAM, with a total of 14,772 children as subjects of the studies. All studies used RCT. Two of them randomized the participants by assigning them to clusters. This finding shows that two updated studies had not been included in the previous review by Schooness *et al.* (2019), which stated that there were eleven studies reporting recovery rate. Studies in this review measured recovery in different ways. Bahwere *et al.* (2014) and Bahwere *et al.* (2017) defined recovery rate as the percentage of children recovered from the study divided by the total number of children who exited the study. Bahwere *et al.* (2016) defined recovery as the absence of bilateral pitting edema and a minimum stay in the program for one month and the case of children admitted with bilateral pitting edema, being clinically well and a MUAC >11.0 cm. Kohlmann *et al.* (2019); Bhandari *et al.* (2017); Jadhav *et al.* (2019); Oakley *et al.* (2010);

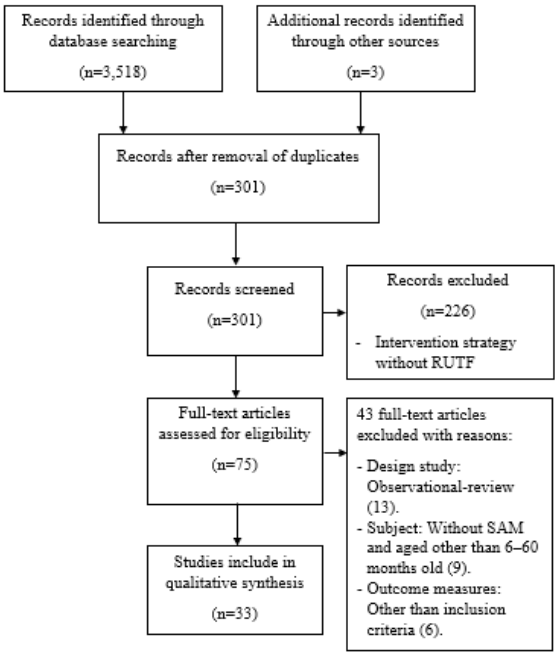

Figure 1. Study flow diagram

Table 1. Studies included in this review

Author (year)	Study design	Number of subjects	Intervention	Outcome measure
Manary *et al.* (2004)	RCT	282	-RUTF -Multivitamin/mineral-fortified RUTF -Maize/soy flour	-Height gain rate -MUAC gain -Weight gain rate
Ciliberto *et al.* (2005)	A controlled, comparative, clinical effectiveness trial	1065	-Home-based therapy with RUTF -Standard therapy	-WHZ -Weight gain rate -Adverse effect
Kerac *et al.* (2009)	RCT	795	-Standard RUTF + synbiotic 2,000 forte -Standard RUTF	-Weight gain rate -Adverse effect
Oakley *et al.* (2010)	RCT (double-blind)	1087	-10% milk RUTF -25% milk RUTF	-Recovery rate -Height gain rate -Weight gain rate
Singh *et al.* (2010)	RCT	112	-Locally produced RUTF -High-calorie cereal milk (HCCM)	-Recovery rate -Blood iron status -Serum albumin -Weight gain rate
Thakur *et al.* (2012)	Quasi trial	98	-L-RUTF: locally made RUTF -F-100	Weight gain rate
Nga *et al.* (2013)	Cross over	67	-Local RUTF (bar) -Plumpy'Nut (paste)	Acceptability
Shewade *et al.* (2013)	RCT	26	-RUTF, supplementary nutrition, feeding counseling, -No-RUTF; only supplementary nutrition and feeding counseling	Weight gain rate
Bahwere *et al.* (2014)	RCT non-inferiority	522	-Milk whey protein-based RUTF -Peanut-based RUTF	Recovery rate
Kaleem *et al.* (2014)	RCT	270	-Imported RUTF -High-Density Diet (HDD) -HDD +micronutrient supplement	-Recovery rate -Weight gain rate
Hsieh *et al.* (2015)	A prospective, randomized, double-blinded, clinical effectiveness trial	141	-Standard RUTF -High oleic acid RUTF	Recovery rate
Irena *et al.* (2015)	RCT (non-blinded)	277	-Peanut-based RUTF -Sorghum-maize-soy RUTF	Weight gain rate

Continue from Table 1

Author (year)	Study design	Number of subjects	Intervention	Outcome measure
Jones *et al.* (2015)	RCT double-blind	61	-Flaxseed oil based-RUTF -Flaxseed oil based-RUTF with addition fish oil capsules -Standard RUTF	Acceptability
Maust *et al.* (2015)	Cluster-RCT	1957	-Integrated management -Standard management	-Recovery rate -Weight gain rate -Adverse effect
Bhandari *et al. (*2016)	Randomized multicenter trial	636	-RUTF-C (centrally produced) -RUTF-L (locally produced) -A-HPF (micronutrient-enriched (augmented) energy-dense home prepared foods)	-Recovery rate -Weight gain rate -Adverse effect
Bahwere *et al.* (2016)	Simple RCT, non-blinded	817	-Dairy-free sorghum-maize-soy RUTF -Peanut-based RUTF	-Recovery rate -Weight gain rate -Mortality rate
Bahwere *et al.* (2017)	A nonblinded, 3-arm, parallel-group simple randomized controlled trial	1075	-Dairy-free, soy, maize and sorghum-based RUTF; -Milk, soy, maize and sorghum-based RUTF -Standard RUTF (peanut and milk–based RUTF)	-Recovery rate -Length of Stay -Weight gain rate -Adverse effect
Ravichandra *et al.* (2017)	RCT	120	-Locally produced RUTF -F-100	-Recovery rate -MUAC gain -Length of stay -Weight-for-length z score -Weight gain rate -Height gain rate
Thapa *et al.* (2017)	RCT	112	-Nutreal RUTF -Defined food	Acceptability
Versloot *et al.* (2017)	RCT (non-blinded)	64	-F100 -F75+RUTF -RUTF	-Length of stay -Adverse effect
Choudhury *et al.* (2018)	A clinical triall with a cross-over design	30	-Rice lentils RUTF -Chickpeas RUTF -Plumpy'Nut	Acceptability
Sato *et al.* (2018)	RCT (non-blinded)	466	-Dairy-free sorghum-maize-soy-RUTF -Milk-sorghum-maize-soy -RUTF -Peanut-milk-RUTF	-Plasma amino acid level -MUAC gain
Sigh *et al.* (2018a)	RCT	75	-NumTrey RUTF -BP100	-Height gain rate -WHZ -HAZ -MUAC gain -Weight gain rate

Continue from Table 1

Author (year)	Study design	Number of subjects	Intervention	Outcome measure
Sigh *et al.* (2018b)	Cross over	52	-NumTrey RUTF -BP100	Acceptability
Akomo *et al.* (2019)	RCT	92	-Dairy-free sorghum-maize-soy RUTF -Milk-sorghum-maize-soy RUTF -Peanut-milk RUTF	-Anemia status -Blood iron status
Kangas *et al.* (2019)	RCT	179	-Reduced RUTF dose -Standard RUTF dose	-Weight gain rate -Height gain rate
Kohlman *et al.* (2019)	RCT, double-blind	1027	-Alternative RUTF -Standard RUTF	-Recovery rate -MUAC gain -Mortality rate -Weight gain rate
Jadhav *et al. (*2019)	An open prospective randomized controlled trial	880	-Indigenous RUTF Medical Nutrition Therapy (MNT) -Standard Nutrition Therapy (SNT)	-Recovery rate -Weight gain rate
Kangas *et al.* (2020)	RCT (non-inferiority study design)	179	-Reduced RUTF dose -Standard RUTF dose	Body composition
Bailey *et al.* (2020)	RCT (cluster) non-inferiority trial	4110	-RUTF treatment with different dose based on MUAC -Standard protocol in each country (RUTF for SAM)	-Recovery rate -Length of stay
Hendrixson *et al.* (2020)	RCT triple blinded	1406	-Oat, peanut and skim milk-based RUTF -Peanut-based RUTF	Weight gain rate
Hossain *et al*. (2020)	Double-blind, randomized non-inferiority trial	120	-Dairy-free soy-based RUTF -Milk-based standard RUTF	-WHZ -MUAC gain -Body composition -Weight gain rate

Bailey *et al.* (2020); Kaleem *et al.* (2014); Singh *et al.* (2010); Maust *et al.* (2015); Hsieh *et al*. (2015); and Ravichandra *et al.* (2017) measured recovery by WLZ >2 SD or MUAC >12.4 cm, WHZ ≥-2 SD and absence of edema, WHZ >-3 SD or MUAC >115 mm, WHZ >-2 SD and no edema, MUAC ≥125 mm and no edema, target weight gain and MUAC ≥11.5 cm, WAZ ≥-2 SD, MUAC >12.4 cm or WHZ ≥-2 SD, had a MUAC >12.4 cm, without edema within 12 weeks of enrollment, attained weight for height Z score of 1 SD below the median of WHO reference and had lost edema for those acute edematous malnutrition, respectively.

Similar to previous reviews (Schooness et *al.* 2019), several studies have compared the effects of giving alternative and standard RUTFs. The others compared the effects of RUTF with F100, energy-dense home-prepared foods, High-Density Diet (HDD) and high-calorie cereal milk (HCCM). The doses of RUTF were 150 kcal/kg/day, 175 kcal/kg/day and 200 kcal/kg/day.

The management of SAM with standard hospital-based protocol proposed by WHO

showed that the recovery rates were around 80% (Bhutta *et al.* 2013; Hossain *et al.* 2020; Khanum *et al.* 1998). The highest result found in this review was shown by Ravichandra *et al.* (2017) where 100% of children given Locally-Prepared RUTF (L-RUTF) recovered, even though they did not mention how long the duration of the intervention was. Most studies did not report the duration of the intervention. According to studies that mentioned the intervention duration, the intervention was carried out for two to four months.

These findings indicates that the management of SAM in children without any medical complications by administering RUTF affects recovery positively than standard protocol in a hospital, even the recovery rate exceeds the SPHERE minimum standards of more than 70% (Bahwere *et al.* 2014). It is in line with previous review by Schooness *et al.* (2019), which revealed that RUTF may improve recovery. Furthermore, a more recent study by Kangas *et al.* (2019) found that reduced dose of RUTF successfully supported recovery in children suffering from SAM without any side effects.

Height gain rate

Linear growth is commonly measured in studies with children with MAM where 2.8 mm/week growth rate was expected in healthy 13-month-old children (WHO 2006). The previous review included four studies that measured this outcome (Schooness *et al.* 2019). However, there was another study conducted in 2010 by Oakley *et al.* All these five studies reported height gain in 2,530 children with SAM with provision of RUTF. Kangas *et al.* (2019) measured height gain after sixteen weeks of intervention and they showed that there was height gain by around 0.63 mm/day with a dose of 1−2 sachets of standard RUTF per day.

In another study by Ravichandra *et al.* (2017) who compared the use of their own Local RUTF formula (L-RUTF) with F-100 formula that is commonly used to treat malnutrition, height gain was measured when participants reached a weight-for-height z score 1 SD below the median. They reported that the height gain of the L-RUTF group was better than that of the F-100 group (0.56 mm vs. 0.42 mm). It shows that locally produced RUTF is as potential as standard RUTF in improving height gain of children with SAM.

Weight-for-height z score (WHZ)

Sigh *et al.* (2018a); Ciliberto *et al.* (2005); and Hossain *et al.* (2020), measured and reported WHZ. A newer study by Hossain *et al.* (2020) evaluated WHZ as an endline value of twelve weeks of intervention. Sigh *et al.* (2018a) also measured WHZ as an endline value and they did not mention how long the intervention was. Another study measured WHZ until the subjects (children) attained a WHZ -2 SD. Among the three studies, the best improvement in WHZ was shown by Hossain *et al.* (2020), where the soy-based RUTF group attained 1.22 of WHZ score and milk-standard RUTF group attained 1.22 of WHZ score. The more significant improvement of WHZ in children receiving home-based therapy with RUTF was achieved because of more rapid weight gain and fewer symptoms of infection happened during their recovery period than in children receiving standard therapy (Ciliberto *et al.* 2005). The time point to measure WHZ among these studies was different. This review's findings are in line with the review by Schoonees *et al.* (2019) indicating that both standard RUTF and locally-produced RUTF may improve WHZ in children with SAM.

Height-for age z score (HAZ)

A study by Sigh *et al.* (2018a), which involved 75 children under five years who experienced SAM, evaluated HAZ after providing interventions including fish-based RUTF (NumTrey) and BP-100 (compressed bar RUTF). They showed no statistically significant difference between the standard RUTF (BP-100) and the alternative RUTF (fish-based RUTF or NumTrey) for HAZ in children under five. This finding indicates that formulation of RUTF with other protein sources, such as fish, might bring positive effect as good as standard formulation that uses milk, since the WHO recommends that at least 50 % of protein should come from milk (WHO/WFP/UNSCN/UNICEF 2007). According to Schoones *et al.* (2019), after eight-week of intervention, there was no significant difference in HAZ between standard RUTF and locally-produced alternative RUTF. Meanwhile, other reviews (Bhutta *et al.* 2008; Gera 2010) did not discuss this outcome. As acute malnutrition is linked to an increased risk of stunting, HAZ is also an important parameter to be considered in the management of SAM.

Mid-upper arm circumference (MUAC) gain

Seven studies reported the measurement of MUAC gain in 6,088 children under five years. All of those studies were randomized controlled trials. Hossain *et al.* (2020) and Ravichandra *et al.* (2017) measured MUAC gain after twelve weeks of intervention and children attained WHZ 1 SD below the median, respectively. The other studies did not include data on the duration of intervention. Based on the seven studies, MUAC gain ranged from 0.42 mm/day to 0.9 mm/day. An updated study by Hossain *et al.* (2020) that compared milk-based RUTF (standard RUTF) with soy-based RUTF (dairy-free alternative RUTF) showed an increase in MUAC at the end of the intervention between two groups, namely 0.9±0.7 and 0.9±0.6 cm, respectively. This finding reinforces the findings by Schooness *et al.* (2019), which stated that there was no significant difference between the standard RUTF group and the locally-produced alternative RUTF. Since MUAC has the advantage of being more sensitive in younger children, measuring MUAC is essential for monitoring the condition of children with SAM (Goossens *et al.* 2012).

Body composition

Two studies included in this review listed body composition as one of the outcomes measured. The total subjects included in those studies were 199 children. This type of outcome was not included in the previous reviews (Schooness *et al.* 2019; Bhutta *et al.* 2008; Gera 2010). An updated study by Hossain *et al.* (2020), which compared dairy-free soy-based RUTF to standard milk-based RUTF after twelve weeks of intervention, showed that the average values of final fat-free mass and fat mass in the soy-based RUTF group were higher than those of the milk-based RUTF group ($p>0.05$). The final total body water was higher in the milk-based RUTF group than in the soy-based RUTF group.

Another study by Kangas *et al.* (2020) compared the effect of providing reduced dose RUTF to standard dose RUTF in children under five years with SAM for sixteen weeks. There was no difference observed in Fat-Free Mass (FFM), Fat Mass (FM) or Fat Mass Index (FMI) between the groups at recovery. Not only depending on the type of diet used, the proportion of different tissue accretion also depends on the nutritional status of the subjects at admission (MacLean & Graham 1980; Radhakrishna *et al.* 2010). Studies that measured body composition indicated that both standard RUTF and locally-produced RUTF may improve body composition of children with SAM without medical complications.

Length of stay (LoS)

Four studies determined length of stay as an outcome measure. Bahwere *et al.* (2017), who compared two alternative RUTFs (Dairy-Free Sorghum-Maize-Soy (F-SMS) RUTF and Milk Sorghum-Maize-Soy (M-SMS) RUTF) to standard RUTF (peanut and milk-based (PM) RUTF), reported that the length of stay of the two alternative RUTFs (FSMS RUTF (n=144); MSMS RUTF (n=144)) was not inferior to the standard RUTF (PM RUTF (n=143)). Ravichandra *et al.* (2017) showed that LoS in children treated with locally-produced RUTF was significantly shorter than that of children treated with F-100 at thirteen and seventeen days, respectively. Versloot *et al.* (2017) reported that there were no differences in the average length of stay among the three feeding strategies (F100, RUTF + F-75, RUTF), which was 7.0 days (SD 3.4). Bailey *et al.* (2020) also revealed that there were no differences in the average length of stay among the two strategies intervention (RUTF treatment with different dose based on MUAC, Standard protocol in each country (RUTF for SAM)) which was 64.5 and 65 days, respectively. WHO's recommended criteria for discharging children from treatment are when they have WHZ or WLZ ≥- 2, or MUAC ≥125 mm and no edema for at least two weeks (WHO 2013). It is similar to the previous review by Schoonees *et al.* (2019), which found no significant differences in terms of length of stay between standard RUTF group and alternative RUTF group. The finding of this review indicates that SAM treatment with locally-produced RUTF was not lower than the standard RUTF in terms of length-of-stay.

Mortality rate

Three studies comparing the alternative RUTF to the standard RUTF, with a total of 3,228 children as subjects, reported mortality rate as one of the outcomes included in their studies. The three studies showed low mortality rate due to treatment of SAM children with RUTF. Bahwere *et al*. (2014), who evaluated the effect of WPC-RUTF (whey protein concentrate-based RUTF)

compared to the standard RUTF (peanut-based RUTF), showed that the mortality rate of the WPC-RUTF group (1.9%) was slightly higher than that of the standard RUTF group (0.8%) on both ITT (Intention-to-Treat) analysis and PP (per-protocol) analysis. A similar result was also reported by Bahwere *et al.* (2017), who compared the effect of giving F-SMS RUTF (Dairy-Free Soy, Maize and Sorghum RUTF) and M-SMS RUTF (Milk, Sorghum, Maize and Soy RUTF) to the standard RUTF. Analysis on ITT and PP showed that the F-SMS RUTF group (2.5%) and M-SMS RUTF group (1.7%) had slightly higher mortality rate than the standard RUTF group (1.3 %). In contrast, Kohlman *et al.* (2019) reported 0.5 % mortality rate in their study that used locally-produced RUTF, which was lower than in the standard RUTF (1.5%). A systematic review by Gera (2010) reported that locally-produced alternative RUTF had mortality rate as low as that of the standard RUTF, which was less than 1%. Although there were three studies that measured mortality rate, a meta-analysis by Schoonees *et al.* (2019) concluded that there was very low-quality evidence in terms of mortality rate in studies that used RUTF.

Anemia and iron status

Studies by Singh *et al.* (2010) and Akomo *et al.* (2019) with a total of 504 children as subjects reported the effect of RUTF on anemia and iron status in children with SAM. Singh *et al.* (2010) who compared the administration of locally-produced milk and peanut-based RUTF with High-Calorie Cereal Milk (HCCM) with doses of 5.5 kcal/g and two serving portions, respectively, found that there was an increase of hemoglobin level in both groups (RUTF group had lower result than HCCM). Although the increase of hemoglobin level was reported, but the percentage of iron content in each treatment food was not reported.

Akomo *et al.* (2019) used alternative F-SMS RUTF (Dairy-Free Soy, Maize and Sorghum RUTF) and M-SMS RUTF (9% Milk, Soy, Maize and Sorghum) as compared with the standard (milk and peanut-based) RUTF. The researchers allowed children to eat RUTF ad libitum. However, this study did not state how long the intervention was performed. The results indicated that although the alternative RUTF used cereals, the prevalence of anemia, iron deficiency and iron deficiency anemia in these treatment groups was lower than the standard RUTF group. This might be due to the high content of casein, whey and calcium found in milk that inhibit iron absorption (Cercamondi *et al.* 2013). Moreover, the alternative RUTFs used higher content of iron and vitamin C to achieve the ratio of the optimum phytic acid to iron molar for iron absorption. This study also suggests that the iron content in the current standard RUTF needs to be increased since it is not enough to improve the anemia status in malnourished children. These two studies demonstrated that provisioning locally-produced RUTF is a feasible intervention to improve anemia status in malnourished children.

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Albumin and amino acid level

The amino acid level was one of the outcomes reported by Sato *et al*. (2018) that used alternative RUTF similar to that of Akomo *et al.* (2019). Sato *et al.* (2018) measured amino acids (methionine, leucine, valine, isoleucine, lysine, phenylalanine, tryptophan, threonine, histidine, BCAA (Branched-Chain Amino Acids), EAA (Essential Amino Acids), and cystine) level in children with SAM after administering 200 kcal/kg/day of alternative RUTF and standard RUTF. The results showed that the EAA plasma concentrations in 6−59 months old children with SAM treated with the alternative RUTF were not less than those of children treated with the standard RUTF. SAM children who obtain adequate protein and amino acids from their diet may get improvement in their MUAC as well as the absence of bilateral pitting edema (Sato *et al.* 2018). The positive effect of RUTF on protein synthesis was also shown by Singh *et al*. (2010) who found higher increase of serum albumin in the RUTF group than the HCCM group.

Another study that supplemented Aromatic Amino Acids (AAAs) on SAM children also showed positive effect with faster rate of protein synthesis after supplementation with 330 mg/kg/day of AAAs as compared with isonitrogenous Alaninesupplementation during recovery phase (Hsu *et al.* 2014). The non-inferior results of EAA plasma, including methionine and cysteine, in both alternative RUTF groups (AAAs and Isonitrogenous Alanine Suplementation) suggested that the recovery from malnutrition under alternative RUTF treatment was possibly due to the achievement of the stable supply of

GSH Glutathione) by maintenance of plasma methionine and cysteine levels. With the positive effects shown in recovery rate and weight gain reported in a previous study by Bahwere *et. al* (2017), this review supports the conclusion that the protein source or RUTF does not influence the effectiveness of the product as long as the amino acid composition is well balanced.

Weight gain rates

The total number of subjects involved in the studies that measured weight gain rate was 12,871. From 21 studies that included weight gain as the outcome, only two studies met the SPHERE Standard in weight gain of 5 g/kg/day (Bahwere *et al.* 2017; Irena *et al.* 2015). Several studies compared RUTF with other nutrition management programs. Six studies reported that RUTF had a better outcome in weight gain compared with several other programs, such as High Calories Cereal Meal (HCCM), maize and soy flour-based supplementary food, RUTF with vitamin supplementation and F-100 formula (Bhandari *et al.* 2016; Jadhav *et al*. 2019; Singh *et al.* 2010; Ravichandra *et al.* 2017; Thakur *et al.* 2012; Manary *et al.* 2004; Shewade *et al.* 2013). Two studies that compared alternative RUTF with standard RUTF showed lower weight gain rates (Hossain *et al.* 2020; Oakley *et al.* 2010). Two other studies showed lower weight gain in RUTF treatment than High-Density Diet (HDD) and standard management programs (Kaleem *et al.* 2014; Maust *et al.* 2015). Those studies showed that RUTF treatment had better outcome in weight gain compared to other treatments.

Other studies that used alternative RUTFs (cereal and legume-based RUTF, reduced dose RUTF) and compared the non-inferiority analysis with the standard milk and peanut-based RUTF showed that alternative RUTFs were not inferior to standard RUTF in weight gain rate (Bahwere *et al.* 2014; Bahwere *et al.* 2016; Bahwere *et al.* 2017; Kangas *et al.* 2019). Five studies showed no differences between alternative RUTFs (cereal, legume and fish-based RUTF, RUTF with symbiotic 2,000 Forte , cereal and legume-based RUTF) and standard RUTF (Sigh *et al.* 2018a; Kerac *et al.* 2009; Irena *et al.* 2015). All these studies used alternative protein sources such as legumes, fish and combination of cereals and legumes to reduce dependency on milk or other dairy products. Potani *et al.* (2021) stated in their review that RUTF containing less dairy could be a lower-cost option for treatment of SAM. Those studies showed that locally-produced alternative RUTF did not have significant difference in terms of weight gain as compared to the standard RUTF.

The results of reducing RUTF dose to weight gain rate were varied. Manary *et al.* (2004) found that provisioning of RUTF to fulfill 33% of energy requirement did not result in a better outcome than the standard dose. While Kangas *et al.* (2019) and Kangas *et al.* (2020) stated that the effect of reduced RUTF dose on the tissue accretion of treated children was not different from the standard treatment. The same result was also found in Jones *et al.* (2015), which concluded that reduced RUTF dose in combined treatment was non-inferior to the standard care that used a higher dose of RUTF. Weight gain results shown by these studies ranged from 0.7 to 9.95 g/kg/day for the alternative RUTFs and from 0.6 to 7.3 g/kg/day for the standard RUTF with approximately 90 days of ntervention. Those studies showed that reduced dose of RUTF did not give significantly different result than the normal dose of RUTF.

All those findings indicate that only few studies achieved the recommended >5 g/kg/day of weight gain and that most of the studies were still under the recommended weight gain rate. The lower weight gain rate could be caused by edema, since children with edema tend to lose weight during treatment because they need a higher energy and protein intake to prevent protein depletion (Bahwere *et al.* 2014).

Adverse effects

Seven studies reported adverse effects (diarrhea) during RUTF intervention (Bahwere *et al.* 2014; Bhandari *et al*. 2016; Manary *et al.* 2004; Kerac *et al.* 2009; Versloot *et al.* 2017; Maust *et al.* 2015; Ciliberto *et al.* 2005). Bhandari *et al.* (2016) reported the number of children having diarrhea during the treatment phase. Another study by Ciliberto *et al.* (2005) measured the number of days of diarrhea per group during the first two weeks of the treatment period and found that children who received RUTF had a similar frequency of diarrhea as compared to those receiving the home-based therapy. Those studies had similar RUTF form and composition, which refer to standard peanut and milk-based RUTF, but Bhandari (2016) used

locally-based ingredients and peanut paste. In the studies by Versloot *et al.* (2017) and Maust *et al.* (2015), diarrhea was recorded to be higher after the intervention with peanut paste-based standard RUTF.

On the other hand, Bahwere *et al.* (2014); Kerac *et al.* (2009); and Manary *et al.* (2004) measured the proportion of children who had diarrhea. The type of RUTF composition used in those studies was slightly different. Bahwere *et al.* (2014) used standard RUTF (peanut and milk-based) with Whey Protein Concentrate (WPC24), while Kerac *et al.* 2009 used standard RUTF (peanut and milk-based) with symbiotic 2,000 forte and Manary *et al.* (2004) used standard RUTF supplemented with 80% maize and 20% soy.

After the intervention, the highest diarrhea incident was found in the study conducted by Versloot *et al.* (2017), which might be associated to the transition phase. Diarrhea was common in all patients irrespective of the diet group, but it was more prevalent during the transition phase, with an average of 48% at the first day of admission ($p<0.05$). The percentage of children with diarrhea did not decrease during the analysis period.

Acceptability of RUTF

Studies by Choudhury *et al.* (2018); Jones *et al.* (2015); Nga *et al.* (2013); Thapa *et al.* (2017); and Sigh *et al.* (2018b) evaluated the acceptability of RUTF, with a total of 426 children as participants. Choudhury *et al.* (2018) reported that alternative RUTFs (chickpea-based RUTF and rice and lentil-based RUTF) were more acceptable than the standard milk and peanut-based RUTF. A similar result was shown by Thapa *et al.* (2017), where 93% of children participated in the study stated that the alternative RUTF (Nutreal) was acceptable for them. On the other hand, Nga *et al.* (2013) reported that the standard RUTF had a higher palatability score than the local RUTF pressed bar (alternative RUTF). This result was similar to the result shown by Sigh *et al*. (2018b) who reported that Numtrey RUTF (alternative RUTF) that was made from wafer roll filled with fish-based paste was less acceptable than BP-100 in taste trial, even though the acceptability of Numtrey RUTF increased in the intervention trial. Jones *et al.* (2015) also stated that they evaluated the acceptability of their RUTF, but unfortunately no data was found in the report.

Most of the studies included in this review showed that RUTF products in paste form had good acceptability in Africa and South Asia. However, a cross-sectional study carried out in Bangladesh (not included in this review) reported that the standard paste-form RUTF did not have good acceptability according to the children caregivers' perception., with the dissatisfaction came from the taste and consistency of the product (Ali *et al*. 2013). Although the study used caregivers' perception instead of the children, it showed that some aspects need to be considered before development of an alternative RUTF, such as the taste should be less sweet and less salty, the consistency should be less sticky and direct instructions should be provided on the packaging. Nga *et al.* (2013) also suggested that the local preference should always be considered in development of an RUTF product. Other forms of RUTF, such as pressed bar and wafer roll filled with paste, may have good acceptability among children (Nga e*t al.* 2013; Sigh *et al.* 2018b).

Limitation of the studies

The studies included in this review lack monitoring on subjects' compliance and the possibility of sharing the provided RUTF with other family members that could affect the measured outcomes. Moreover, different studies had differences in definition of recovery, cut-off points for each outcome, doses that were used, and duration of interventions that might affect the outcomes. In terms of food safety, majority of the studies did not report any microbiological tests performed or if there were any cases of diarrhea among subjects. Diarrhea is considered one of the important side effects, which may not only happen during transition phase, but it also may recur during rehabilitation phase

CONCLUSION

Standard peanut and milk-based RUTF is used in most of the studies included in this review. Nine out of twenty-two studies showed that standard RUTF had positive effects on recovery rate of SAM children. When alternative RUTF formulas were developed from locally-available ingredients such as fish (whole fish, fish oil), legumes (soy bean, mung bean, chick peas,

lentils) and cereals (maize, rice, oats, sorghum), each of them showed variety of strengths in terms of outcomes measured, for example improvement in anemia status from administration of RUTF with combination of cereals and legumes, improvement of blood PUFA status in subjects treated with RUTF added with fish oil, and various other improvements in other RUTF formulas. In general, locally produced RUTF showed positive effects on recovery rate of under five years old children with SAM, with no statistically significant differences with the standard RUTF. It should be noted that compliance to the study protocols must be recorded since it may affect conclusions made.

The studies that were reviewed showed that locally produced RUTF has potentials, providing that the researchers include sensory test as an important criterion to ensure better acceptance from the subjects. Special attention should also be paid when fish is included in the formula, since its flavor is not widely accepted. Furthermore, the form of the product was also found to influence sensory acceptance from the subjects. Therefore, development of alternative RUTFs should always consider local preferences, ingredients availability, production sustainability and product safety (hygiene and sanitation).

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DECLARATION OF INTERESTS

Rimbawan Rimbawan (R R), Zuraidah Nasution (Z N) and Puspo Edi Giriwono (P E G) conceptualized the idea and methodology followed by reviewing and editing. Kharisma Tamimi (K M), Khaerul Fadly (K F) and Astrid Noviana (A N) screened resources and writing original draft. All authors have read and agreed to the published version of the manuscript.

REFERENCES

Akomo P, Bahwere P, Murakami H, Banda C, Maganga E, Kathumba S, Sadler K, Collins S. 2019. Soya, maize, and sorghum ready-to-use-therapeutic foods are more effective in correcting anemia and iron deficiency than the standard ready-to-use therapeutic food: Randomized controlled trial. BMC Pub Health 19(1):806. https://doi.org/10.1186/s12889-019-7170-x

Ali E, Zachariah R, Dahmane A, Van den Boogaard W, Shams Z, Akter T, Alders P, Allaouna M, Draguez B, Delchevalerie P, Harries AD. 2013. Peanut-based ready-to-use therapeutic food: Acceptability among malnourished children and community workers in Bangladesh. Public Health Action 3(2):128−135. https://doi.org/10.5588/pha.12.0077

Bahwere P, Banda T, Sadler K, Nyirenda G, Owino V, Shaba B, Dibari F, Collins S. 2014. Effectiveness of milk whey protein-based ready-to-use therapeutic food in the treatment of severe acute malnutrition in Malawian under-5 children: A randomized, double-blind, controlled non-inferiority clinical trial. Maternal & Child Nutr 10(3):436−451. https://doi.org/10.1111/mcn.12112

Bahwere P, Balaluka B, Wells JCK, Mbiribindi CN, Sadler K, Akomo P, Dramaix-Wilmet M, Collins S. 2016. Cereals and pulse-based ready-to-use therapeutic food as an alternative to the standard milk- and peanut paste-based formulation for treating severe acute malnutrition: A non-inferiority, individually randomized controlled efficacy clinical trial. Am J Clin Nutr 103(4):1145−1161. https://doi.org/10.3945/ajcn.115.119537

Bahwere P, Akomo P, Mwale M, Murakami H, Banda C, Kathummba S, Banda C, Jere S, Sadler K, Collins S. 2017. Soya, maize, and sorghum-based ready-to-use therapeutic food with amino acid is as efficacious as the standard milk and peanut paste-based formulation for the treatment of severe acute malnutrition in children: A non-inferiority individually randomized controlled efficacy clinical trial in malawi. Am J Clin Nutr 106(4):1100−1112. https://doi.org/10.3945/ajcn.117.156653

Bailey J, Opondo C, Lelijveld N, Marron B, Onyo P, Musyoki EN, Adongo SW, Manary M, Briend A, Kerac M. 2020.

A simplified, combined protocol versus standard treatment for acute malnutrition in children 6–59 months (ComPAS trial): A cluster-randomized controlled non-inferiority trial in Kenya and South Sudan. PLoS Med 17(7):e1003192. https://doi.org/10.1371/journal.pmed.1003192

Bhandari N, Mohan SB, Bose A, Iyengar SD, Taneja S, Mazumder S, Pricilla RA, Iyengar K, Sachdev HS, Mohan VR, Suhalka V. 2016. Efficacy of three feeding regimens for home-based management of children with uncomplicated severe acute malnutrition: A randomized trial in India. BMJ Glob Health 1(4):e000144. https://doi.org/10.1136/bmjgh-2016-000144

Bhutta ZA. Ahmed T, Black RE, Cousens S, Dewey K, Giugliani E, Haider BA, Kirkwood B, Morris SS, Sachdev HP *et al.* 2008. What works? Interventions for maternal and child undernutrition and survival. The Lancet 371(9610):417–440. https://doi.org/10.1016/S0140-6736(07)61693-6

Bhutta ZA, Das JK, Rizvi A, Gaffey MF, Walker N, Horton S, Webb P, Lartey A, Black RE. 2013. Evidence-based interventions for improvement of maternal and child nutrition: What can be done and at what cost? Lancet 382(9890):452−477. https://doi.org/10.1016/S0140-6736(13)60996-4

Cercamondi Cl, Egli IM, Mitchikpe E, Tossou F, Hessou J, Zeder C, Hounghouigan JD, Hurrek RF. 2013. Iron bioavailability from a lipid-based complementary food fortificant mixed with millet porridge can be optimized by adding phytase and ascorbic acid but not using a mixture of ferrous sulfate and sodium iron EDTA. J Nutr 143(8):1233−1239. https://doi.org/10.3945/jn.113.175075

Choudhury N, Ahmed T, Hossain MI, Islam MM, Sarker SA, Zeilani M, Clemens JD. 2018. Ready-to-use therapeutic food made from locally available food ingredients is well accepted by children having severe acute malnutrition in Bangladesh. Food Nutr Bull 39(1):116−126. https://doi.org/10.1177/0379572117743929

Ciliberto MA, Sandige H, Ndekha MJ, Ashorn P, Briend A, Cilliberto HM, Manary MJ. 2005. Comparison of home-based therapy with ready-to-use therapeutic food with standard therapy in the treatment of malnourished malawian children: A controlled, clinical effectiveness trial. Am J Clin Nutr 81(4):864−870. https://doi.org/10.1093/ajcn/81.4.864

Codex Alimentarius Commission. 2019. Joint FAO/WHO Foods Standards Programme, Codex Committe on Nutrition and Foods for Special Dietary Uses, Proposed Draft Guideline for Ready-to-use Therapeutic Foods. Rome (IT): Food and Agriculture Organization.

De Pee S, Bloem MW. 2009. Current potential role of specially formulated foods and food supplements for preventing malnutrition among 6−23 months old children and treating moderate malnutrition among 6−59 months old children. Food Nutr Bull 30(3):1−40. https://doi.org/10.1177/15648265090303S305

Gera T. 2010. Efficacy and safety of therapeutic nutrition products for home-based therapeutic nutrition for severe acute malnutrition: A systematic review. Indian Pediatr 47(8):709−718. https://doi.org/10.1007/s13312-010-0095-1

Goossens A, Bekel Y, Yun O, Harczi G, Ouannes M, Shepherd S. 2012. Mid-upper arm circumference based nutrition progrmming: Evidence for a new approach in regions with high burden of acute malnutrition. PLoS One 7(11):e49320. https://doi.org/10.1371/journal.pone.0049320

Hendrixson DT, Godbout C, Los A, Callaghan-Gillespie M, Mui M, Wegner Dm Bryant T, Koroma A, Manary MJ. 2020. Treatment of severe acute malnutrition with oat or standard ready-to-use therapeutic food: A triple-blind, randomized controlled clinical trial. Gut 29(12):1−7. https://doi.org/10.1136/gutjnl-2020-320769

Hossain MI, Huq S, Islam MM, Ahmed T. 2020. Acceptability and efficacy of ready-to-use therapeutic food using soy protein isolate in under-5 children suffering from severe acute malnutrition in Bangladesh: a double-blind randomized non-inferiority trial. Eur J of Nutr 59(3):1149−1161. https://doi.org/10.1007/s00394-019-01975-w

Hsieh JC, Liu L, Zeilani M, Ickes S, Trehan I, Maleta K, Craig C, Thakwalakwa C, Singh L, Brenna JT *et al.* 2015. High-oleic

ready-to-use therapeutic food maintains docosahexaenoic acid status in severe malnutrition. J Pediatr Gastroenterol Nutr 61(1):138−143. https://doi.org/10.1097/MPG.0000000000000741

Hsu JW, Badaloo A, Wilson L, Taylor-Bryan C, Chambers B, Reid M, Forrester T, Jahoor F. 2014. Dietary supplementation with aromatic amino acids increases protein synthesis in children with severe acute malnutrition. J Nutr 144(5):660−666. https://doi.org/10.3945/jn.113.184523

Irena AH, Bahwere P, Owini VO, Diop EHI, Bachmann MO, Mbwili-Muleya C, Dibari F, Sadler K, Collins S. 2015. Comparison of the effectiveness of a milk-free soy-maize-sorghum-based ready-to-use therapeutic food to standard ready-to-use therapeutic food with 25% milk in nutrition management of severely acutely malnourished Zambian children: An equivalence non-blinded cluster randomized controlled trial. Maternal and Child Nutr 11(4):105−119. https://doi.org/10.1111/mcn.12054

Jadhav AR, Karnik P, Fernandes L, Fernandes S, Shah N, Manglani M. 2019. Indigenously prepared ready-to-use therapeutic food (RUTF) in children with severe acute malnutrition. Indian Pediatr 56(4):287−293. https://doi.org/10.1007/s13312-019-1516-4

Jones KD, Ali R, Khasira MA, Odera D, West AL, Koster G, Akomo P, Talbert WA, Goss VM, Ngari M *et al.* 2015. Ready-to-use therapeutic food with elevated n-3 polyunsaturated fatty acid content, with or without fish oil, to treat severe acute malnutrition: A randomized controlled trial. BMC Medi 13(1):1−14. https://doi.org/10.1186/s12916-015-0315-6

Kaleem R, Aziz N, Zaman S, Salimi MA. 2014. Nutritional rehabilitation of severely malnourished children by high density diet in comparison to ready to use therapeutic food. Pak Pediatr J 38(4):235−243.

Kangas ST, Salpéteur C, Nikièma V, Talley L, Ritz C, Friis H, Briend A, Kaestel P. 2019. Impact of reduced dose of ready-to-use therapeutic foods in children with uncomplicated severe acute malnutrition: A randomized non-inferiority trial in Burkina Faso. Plos Med 16(8):e1002887. https://doi.org/10.1371/journal.pmed.1002887

Kangas ST, Kaestel P, Salpéteur, C, Nikièma V, Talley L, Briend A, Ritz C, Friis H, Wells JC. 2020. Body composition during outpatient treatment of severe acute malnutrition: Results from a randomized trial testing different doses of ready-to-use therapeutic foods. Clinical Nutr 39(11):3426−3433. https://doi.org/10.1016/j.clnu.2020.02.038

Kerac M, Bunn J, Seal A, Thindwa M, Tomkins A, Sadler K, Bahwere P, Collins S. 2009. Probiotics and prebiotics for severe acute malnutrition (PRONUT study): A double-blind efficacy randomized controlled trial in Malawi. Lancet 373(9684):136−144. https://doi.org/10.1016/S0140-6736(09)60884-9

Khanum S, Ashworth A, Huttly SR. 1998. Growth, morbidity, and mortality of children in Dhaka after treatment for severe acute malnutrition: A prospective study. Am J Clin Nutr 67(5):940−945. https://doi.org/10.1093/ajcn/67.5.940

Kohlmann K, Callaghan-Gillespie M, Gauglitz JM, Steiner-Asiedu M, Saalia K, Edwards C, Manary MJ. 2019. Alternative ready-to-use therapeutic food yields less recovery than the standard for treating acute malnutrition in children from ghana. Global Health Sci and Prac 7(2):203−214. https://doi.org/10.9745/GHSP-D-19-00004

[MoH RI] Ministry of Health of Republic of Indonesia. 2018. The Primary Outcome of Basic Health Research 2018. Jakarta (ID): Ministry of Health of Republic of Indonesia.

MacLean WC, Graham GG. 1980. The effect of energy intake on nitrogen content of weight gain by recovering malnourished infants. Am J Clin Nutr 33(4):903−909. https://doi.org/10.1093/ajcn/33.4.903

Manary MJ, Ndekha MJ, Ashorn P, Maleta K, Briend A. 2004. Home based therapy for severe malnutrition with ready-to use food. Arch Dis child 89(6):557−561. https://doi.org/10.1136/adc.2003.034306

Maust A, Koroma AS, Abla C, Molokwu N, Ryan KN, Singh L, Manary MJ. 2015. Severe and moderate acute malnutrition can be successfully managed with an integrated protocol in Sierra Leone. J

Nutr 145(11):2604−2609. https://doi.org/10.3945/jn.115.214957

Nga TT, Nguyen M, Mathisen R, Hoa DT, Minh NH, Berger J, Wieringa FT. 2013. Acceptability and impact on anthropometry of a locally developed ready-to-use therapeutic food in pre-school children in Vietnam. Nutr J 12(1):1−8. https://doi.org/10.1186/1475-2891-12-120

Oakley E, Reinking J, Sandige H, Trehan I, Kennedy G, Maleta K, Manary M. 2010. A ready-to-use therapeutic food containing 10% milk is less effective than one with 25% milk in the treatment of severely malnourished children. J Nutr 140(12):2248−2252. https://doi.org/10.3945/jn.110.123828

Potani I, Spiegel-Feld C, Brixi G, Bendabenda J, Siegfried N, Bandsma RH, Briend A, Daniel AI. 2021. Ready-to-use therapeutic food (RUTF) containing low or no dairy compared to standard RUTF for children with severe acute malnutrition: A systematic review and meta-analysis. Adv Nutr 12(5):1930−1943. https://doi.org/10.1093/advances/nmab027

Radhakrishna KV, Kulkarni B, Balakrishna N, Rajkumar H, Omkar C, Shatrugna V. 2010. Composition of weight gain during nutrition rehabilitation of severly under nourished children in a hospital based study from India. Asia Pac J Clin Nutr 19(1):8−13. https://search.informit.org/doi/10.3316/ielapa.964169765094053

Ravichandra KR, Bahera N. 2017. Locally prepared ready to use therapeutic food for the treatment of children with severe acute malnutrition: A randomized controlled trial. Int J Contemp Pediatr 4(4):1491−1495. https://doi.org/10.18203/2349-3291.ijcp20172692

Sato W, Furuta C, Matsunaga K, Bahwere P, Collins S, Sadler K, Akomo P, Banda C, Manganga E, Kathumba S *et al.* 2018. Amino-acid-enriched cereals ready-to-use therapeutic foods (RUTF) are as effective as milk-based RUTF in recovering essential amino acid during the treatment of severe acute malnutrition in children: An individually randomized control trial in Malawi. PloS ONE 13(8):e201686. https://doi.org/10.1371/journal.pone.0201686

Schoonees A, Lombard MJ, Musekiwa A, Nel E, Volmink J. 2019. Ready-to-use therapeutic food (RUTF) for home-based nutritional rehabilitation of severe acute malnutrition in children from six months to five years of age. Cochrane Database Syst Rev 5:1−178. https://doi.org/10.1002/14651858.CD009000.pub3

Shewade HD, Patro BK, Bharti B, Soundappan K, Kaur A, Taneja N. 2013. Effectiveness of indigenous ready-to-use therapeutic food in community-based management of uncomplicated severe acute malnutrition: A randomized controlled trial from India. J Tropical Pediatrics 59(5):393−398. https://doi.org/10.1093/tropej/fmt039

Sigh S, Roos N, Chamnan C, Laillou A, Prak S, Wieringa FT. 2018a. Effectiveness of a locally produced, fish-based food product on weight gain among Cambodian children in the treatment of acute malnutrition: A randomized controlled trial. Nutrients 10(7):909. https://doi.org/10.3390/nu10070909

Sigh S, Roos N, Sok D, Borg B, Chamnan C, Laillou Am Dijkhuizen MA, Wieringa FT. 2018b. Development and acceptability of locally made fish-based for the ready-to-use therapeutic products for the prevention and treatment of malnutrition in Cambodia. Food Nutr Bull 39(3):420−434. https://doi.org/10.1177/0379572118788266

Singh AS, Kang G, Ramachandran A, Sarkar R, Peter P, Bose A. 2010. Locally made ready-to-used therapeutic food for treatment of malnutrition: A randomized controlled trial. Indian Pediatr 47(8):679−686. https://doi.org/10.1007/s13312-010-0100-8

Thakur GS, Singh HP, Patel C. 2012. Locally-prepared ready-to-use therapeutic food for children with severe acute malnutrition: a controlled trial. Indian Pediatr 50(3):295−299. https://doi.org/10.1007/s13312-013-0097-x

Thapa BR, Goyal P, Menon J, Sharma A. 2017. Acceptability and efficacy of locally produced ready-to-use therapeutic food nutreal in the management of severe acute malnutrition in comparison with defined food: A randomized control trial. Food Nutr Bull 38(1):18−26. https://doi.org/10.1177/0379572116689743

Versloot CJ, Voskuijil W, Van Vliet SJ, van den Heuvel M, Carter JC, Phiri A, Kerac M, Heikens GT, Rheenen PF, Bandsma HJ. 2017. Effectiveness of three commonly used transition phase diets in the inpatient management of children with severe acute malnutrition: A pilot randomized controlled trial in Malawi. B M C Pediatr 17(1):1−8. https://doi.org/10.1186/s12887-017-0860-6

Victoria CG, Adair L, Fall C. 2008. Maternal and child undernutrition: Consequences for adult health and human capital. Lancet 371(9609):302−302 https://doi.org/10.1016/S0140-6736(07)61692-4

[WHO] World Health Organization. 2006. WHO Child Growth Standards: Length/Height-For-Age, Weight-For-Age, Weight-For-Length, Weight-For-Height and Body Mass Index-For-Age: Methods and Development. Geneva (CH): World Health Organization.

[WHO] World Health Organization. 2013. Guideline Update on the Management of Severe Acute Malnutrition in Infants and Children. Geneva (CH): World Health Organization.

[WHO/WFP/UNSCN/UNICEF] World Health Organization, World Food Programme, United Nations System Standing Committee on Nutrition & United Nations Children's Fund. 2007. Community-based management of severe acute malnutrition: A joint statement by the World Health Organization, the World Food Programme, the United Nations System Standing Committee on Nutrition and the United Nations Children's Fund. World Health Organization. https://apps.who.int/iris/handle/10665/44295 [Accessed 6th May 2022].

Young J, Solomon M. 2009. How to critically appraise an article. Nat Rev Gastroenterol Hepatol 6(2):82−91. https://doi.org/10.1038/ncpgasthep1331