

EATING BEHAVIOR OF INDONESIAN ADULTS DIFFER BY METROPOLIZATION LEVELS BASED ON THE 2018 INDONESIAN FOOD BAROMETER

Perbedaan Perilaku Makan Orang Dewasa di Indonesia pada Berbagai Level Metropolisasi berdasarkan Indonesian Food Barometer 2018

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ABSTRACT

Modernization leads to nutrition transition process, shifts eating behavior into a more modernized pattern that may increase the risk of non-communicable diseases (NCDs). Using the 2018 Indonesian Food Barometer (IFB) data, this study assessed the differences in eating behavior between metropolization levels among Indonesian adults. 1482 adults aged 18-64 in six provinces were involved. Eating behavior was defined by the number of eating events, food preparation, eating location, eating companion, and activity while eating. Metropolization level was defined based on the gross domestic regional product (GRDP), GRDP/capita, population size, population density, and non-agricultural worker proportion. Differences in each eating behavior dimension between metropolization levels were analyzed using the Chi-square test ($p < 0.05$) and adjusted with sociodemographic factors using logistics regression. 83.9% of the subjects had ≤ 5 eating events/day and dominated by 4-5 eating events/day. 51.2% and 83.1% of the subjects cooked and ate at home frequently, indicating the practice of traditional eating. Meanwhile, the tendency towards modern dimension can be seen as the proportion of eating alone frequently and eating only less frequently were 60.3% and 67.9%, respectively. Eating behavior differed significantly ($p < 0.05$) between metropolization levels, except for eating with company. Proportion of not cooking and eating away from home frequently increase as metropolization level increases ($p < 0.05$) after adjusted with sociodemographic factors while eating alone and eating only showed no significant differences. Modern eating behavior tendency increases along with metropolization level. Improving living and working conditions in metropolitan areas is imperative to facilitate healthier eating behavior among the inhabitants.

Keywords: adults, eating behavior, metropolitan, modern, nutrition transition

INTRODUCTION

Modernization, urbanization, economic development, and increased wealth led to a shift in dietary patterns called the nutrition transition process (Sannigrahi, 2016), which was indicated among others by the increase of overweight and obesity (Popkin & Ng, 2022). Indonesian Basic Health Research (*Riset Kesehatan Dasar/Riskesdas*) 2018 showed that overweight prevalence among adults gradually increased from 8.6% in 2007 to 11.5% in 2013 and 13.6% in 2018. A similar pattern was also observed in obesity prevalence, with urban areas showing higher prevalence than rural areas. Higher consumption of high-risk food,

such as sweet foods and beverages, salty foods, fried/fatty foods, and processed animal protein, as well as an increase of sedentary behavior in urban areas might explain this phenomenon (Balai Penelitian dan Pengembangan Kesehatan, 2019).

Modernization involves a series of sociodemographic factors such as urbanization or more precisely metropolization, increased purchasing power, level of education, number of individuals per household and stylization of consumption (Poulain et al., 2015). As an area becomes more industrialized, the urbanization process follows. Advanced urbanization transforms cities into metropolitan areas. Indonesian

geographical complexities as the biggest archipelago resulted in the different growth rates of urban areas. Based on the demographic indicator, main cities in different regions in Indonesia showed varied levels of metropolization. Jakarta as the largest metropolitan city in Indonesia has approximately ten million population, Surabaya as the second has 4-5 million population while Medan as the biggest city in Sumatera, and Makassar as the biggest in the eastern area have two million and 1.5 million population, respectively (Adisasmita, 2013).

As the cities grow, increased socioeconomic advantages may affect the health and well-being of the population. Evidence showed that increasing urbanization is in line with increasing health risks. Higher urbanicity and higher education level in the area may lead to more sedentary occupational physical activity (Khusun et al., 2016). The traditional eating pattern is gradually replaced with modern eating as people are adjusting to modernization dynamics. Modern eating can be seen from the shift from a regular food day pattern to 'grazing' (repetitive and unplanned consumption of small amount of food), eating together to eating alone, buying meals instead of cooking, and others (Sproesser et al., 2019). Modern eating practice can be seen from the simplification of meal structures, the increase of in-between meals, the location of food consumption, and profiles of food days (Poulain, 2017).

Increased urbanization and income in low and middle-income countries (LMICs) was in line with the adoption of urban lifestyles, such as eating out and consumption of food prepared away from home (Zhai et al., 2014). A study by Mognard et al. (2023) showed that in 50% of meals were purchased out of home in Singapore, while 35% and 21% of lunch in Indonesia and Malaysia were eaten outside of home. Urban population has been shown to exhibit a high proportion of eating out habit, among others due to food environments and social patterns of eating. In urban slum area, frequencies of buying food from small shops (*warung*), street food vendors, and modern food stores, which are abundant around the neighborhood, were significantly associated with consumption of snacks, mixed dishes, and fruit (Anggraini et al., 2016). The consumption of food considered as 'less healthy pattern' such as

fried foods, oils, and fats was associated with higher risk of obesity (Khusun et al., 2016). The shift of dietary pattern shifted the prevalence of obesity towards the lower socioeconomic population (Yulia et al., 2016). Eating location, especially leisure places, food outlets, and on-the-go, was associated with increased consumption of non-core food, such as pastries, high-fat snacks, and sugary beverages (Ziauddeen et al., 2017), frequent eating alone was found to influence the consumption of sweet snacks and beverages, processed foods (Kwon et al., 2018), and longer eating duration within a day were related to increased fat intake (Tiuganji et al., 2020). Social patterns of eating might explain the overweight/obesity pandemic (Bittman et al., 2019).

The 2018 Indonesian Food Barometer (IFB) was part of the Asian Food Barometer (AFB), the tools developed to study the socioeconomic, demographic, and cultural determinants of food consumption and to analyze the health and sociocultural consequences of nutrition transition and food transition (Khusun, et al., 2022). Cultural and social dimensions of eating cannot be ignored, seeing their impact on decision-making regarding food intake, which will influence health outcomes (Briones Alonso et al., 2018); Robinson & Field, 2015). Most existing studies focused on what people eat, such as the type and the amount of food consumed, while subdimensions of how people eat remain under-explored.

As a rapidly developing country, more cities in Indonesia will grow into metropolitan areas. More than 70% of Indonesians are productive adults who play crucial roles in economic growth (Peterson, 2017) and the caretakers of the next generation. Considering the urgency, this study will explore the differences in eating behavior between metropolization levels among Indonesian adults based on the 2018 IFB.

METHODS

Ethical approval was issued by the Ethical Committee Faculty of Medicine, Universitas Indonesia – Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia, with the number KET-446/UN2.F1/ETIK/PPM.00.02/2023. This study is an exploratory quantitative study with a cross-sectional study design conducted in December 2022 until

April 2023 using secondary data from the 2018 IFB. IFB data was collected in March-July 2018 and involved adults aged >18 years old in six provinces in Indonesia (Jakarta, East Java, West Java, South Sulawesi, Bali, and West Sumatera covering both urban and rural areas. Subjects were selected using multi-stage random sampling, proportionate to population size, to obtain a representative sample of the Indonesian population. Sociodemographic characteristics data and dietary intake were collected using a structured questionnaire 24-h food recall, respectively, through in-person interviews (Khusun et al., 2022)

The current study only included non-pregnant/lactating productive adults (18-64 years old). From the total of 1665 subjects of the 2018 IFB, the current study included 1482 subjects. Metropolization was defined based on the gross domestic regional product (GRDP), GRDP/capita, population size, population density, and proportion of workers in the non-agricultural sector (Dardak et al., 2008). Cities/municipalities were ranked for each criterion, and weights were applied on each criterion according to its contribution to metropolization. Total score was calculated and metropolization was sorted into four levels from the least metropolized to the most metropolized: non metropolitan (Padang Pariaman, Luwu Timur, and Klungkung), semi metropolitan (Garut, Lumajang, and Denpasar), secondary metropolitan (Bandung, Makassar, and Padang), and primary metropolitan (DKI Jakarta, and Surabaya).

Eating behavior was defined by the number of eating events, food preparation, eating location, eating companion, and activity while eating. The number of eating events was classified as ‘≤5 eating events’ and ‘>5 eating events’ which comprised any eating events, including meals and snacks. Since the number of eating events differed between subjects, the other eating behavior dimensions were defined based on the relative proportion of each activity reported from the number of eating events. The relative proportion was then recategorized as ‘less frequent’ (≤66.67%) and ‘frequent’ (>66.67%) (Holm et al., 2016). The differences in each eating behavior dimension between metropolization levels and sociodemographic factors were analyzed using the Chi-square test (p<0.05). Significant results with p<0.02 were analyzed further using logistics

regression. Data were analyzed using IBM SPSS Statistics version 29.

RESULTS AND DISCUSSIONS

The number of subjects included in this study was 1482 subjects with the majority being 26-35 years old (29.9%), male (51.5%), Jawa-Madura (24.6%), in the highest income tertile (49.3%), had graduated from junior and/or high school (62.6%), workers (60.3%), and lived in small households (50.0%) (Table 1). The sociodemographic characteristics of the subjects in this study reflects the characteristics of Indonesian adults in 2018. Central Bureau of Statistics (BPS) data showed that most Indonesian adults have a middle income, which might result from the different standards used to determine income level (Badan Pusat Statistik, 2018) The result of this study can still be generalized to a similar population.

Table 1. Sociodemographic Characteristics of the Subjects

Variable	n (%)
Age	
18-25 y.o.	328 (22.1%)
26-35 y.o.	443 (29.9%)
36-45 y.o.	321 (21.7%)
>45 y.o.	390 (26.3%)
Sex	
Male	763 (51.5%)
Female	719 (48.5%)
Ethnicity	
Sunda	284 (19.2%)
Betawi	117 (7.9%)
Jawa-Madura	364 (24.6%)
Bali	270 (18.2%)
All Sumatra ^a	242 (16.3%)
Sulawesi ^b	205 (13.8%)
Income^c	
T1	511 (34.5%)
T2	241 (16.3%)
T3	730 (49.3%)
Education^d	
Low	333 (22.5%)
Middle	927 (62.6%)
High	222 (15.0%)
Occupation^e	
Workers	894 (60.3%)
Non-workers	203 (13.7%)

Housewives	385 (26.0%)
Family size^f	
Single-person HH	44 (3.0%)
Small HH	741 (50.0%)
Large HH	697 (47.0%)
Metropolization Level^g	
Non-metropolitan	369 (24.9%)
Semi metropolitan	390 (26.3%)
Secondary metropolitan	365 (24.6%)
Primary metropolitan	358 (24.2%)

^aComprises all ethnicity in Sumatra region

^bComprises all ethnicity in Sulawesi region

^cIncome variable was developed from composite measures of household cumulative living standard analysed using principal component analysis (PCA) and defined as tertile with T1 as lowest income, T2 as middle income, and T3 as highest income level

^dEducation level was defined based on the highest educational attainment of the subjects; ‘low education’ comprises subjects with elementary school to lower educational attainment, ‘middle education’ consists of subjects graduated from junior and/or high school, and ‘high education’ consists of subjects with diploma or higher educational attainment.

^e‘Workers’ including all type of workers, ‘non-workers’ comprises of unemployed subjects, students, and retirees

^fFamily size was classified based on the number of people living together with the subjects; Single person household consists of subjects who live alone, small household comprised of family with up to 4 members, while families with >4 family members

^gMetropolization level was defined using data from BPS; ‘Non metropolitan’ consists of Padang Pariaman, Luwu Timur, Klungkung, ‘semi metropolitan’ consists of Denpasar, Garut, Lumajang, ‘secondary metropolitan’ consists of Bandung, Makassar, Padang, and ‘primary metropolitan’ consists of DKI Jakarta and Surabaya

Eating behavior of the subjects showed the practice of traditional eating patterns as seen from the number of eating events, food preparation, and eating location dimensions. 83.9% of the subjects had ≤5 eating events in a day. This study found that the subjects had at least one eating event and a maximum of 10 eating events within a day. A higher proportion of >5 eating events in a day was observed in more metropolized areas (primary and secondary metropolitan areas). A higher number of eating events may indicate ‘grazing’ behavior as the influence of modernization (Sproesser et al., 2018). ‘Grazing’ pattern was found to be associated with higher energy intake and later night-time eating which increase the risk of metabolic diseases (Manoogian et al., 2019; Zeballos & Chelius, 2021).

Most of the subjects prepared food by cooking and eating at home, showing the practice of traditional eating behavior dimension. However,

modern eating practices were observed in the eating companion and activity while eating in which eating alone frequently and eating only less frequently were more dominant (60.3% and 67.9%). (Table 2).

Table 2. Distribution of Eating Behavior in a Day among Subjects (N=1482)

Variable		n (%)	
Number of eating events ^h	≤5 eating events	1244	(83.9%)
	>5 eating events	238	(16.1%)
Food preparation ⁱ	Cooking	Less Frequent ^j	723 (48.8%)
		Frequent	759 (51.2%)
	Not cooking	Less Frequent	1150 (77.6%)
		Frequent	332 (22.4%)
Eating location	Home	Less Frequent	251 (16.9%)
		Frequent	1231 (83.1%)
	Away from home	Less Frequent	1376 (92.8%)
		Frequent	106 (7.2%)
Eating companion	With company	Less Frequent	1167 (78.7%)
		Frequent	315 (21.3%)
	Alone	Less Frequent	589 (39.7%)
		Frequent	893 (60.3%)
Activity while eating	Eating only	Less Frequent	1007 (67.9%)
		Frequent	475 (32.1%)
	Eating while doing something else	Less Frequent	762 (51.4%)
		Frequent	720 (48.6%)

^hNumber of eating events were calculated based on total number of eating events among the subjects within a day and categorized as up to 5 total number of eating events within a day and more than 5 total number of eating events within a day

ⁱFood preparation was defined as the way the subjects prepare their food. Cooking and cooked by friends/partners were categorized as ‘cooking’ while means other than cooking such as buying, catered, delivery, or given by someone else were categorized as ‘not cooking’

^j‘Less frequent’ and ‘frequent’ categories were developed based on the relative proportion from total number of eating events of each eating behavior dimension. The proportion up to 66.67% then recategorized as ‘less frequent’ and proportion above 66.67% were categorized as ‘frequent’

Table 3 shows that after adjustment with sociodemographic factors (age, sex, ethnicity, income, education, occupation, and family size), significant differences were still observed in eating behavior dimensions (except on eating alone and eating only) between metropolization levels. Within the number of eating events, the highest tendency to eat >5 times was observed in the

secondary metropolitan area (aOR 2.303; 1.439–3.686). Metropolitan residents usually engage with modernized lifestyles and habits. Modernization contributes to the increased number of eating events as it influences people to no longer follow fixed eating pattern and ‘grazing’ becomes more common (Sproesser et al., 2018). In addition, the disruption of circadian rhythm from electricity and light usage results in longer waking time, which could lead to irregular eating since there is a longer eating period within a day (Paoli et al., 2019; J. P. Poulain et al., 2020).

The tendency towards modern eating was found in food preparation and eating location as aOR value for modernized eating behavior (not cooking and eating away from home) increased as the metropolization levels increased. This finding was consistent with the traditional eating behavior (cooking and eating at home) that decreased as the metropolization levels increased. Areas with higher urbanization levels are more spatially and financially stable and have more varied food outlets which may affect their food system in general. Practical food preparation methods, such as buying food instead of cooking, are popular among modern eaters (Sproesser et al., 2018), as a coping mechanism for time constraints (Tharrey et al., 2020).

Eating alone practice showed no significant patterns between metropolization levels after adjustment with sociodemographic factors. Activity while eating showed higher odds (1.880; 1.212–2.914) of eating while doing something else in the primary metropolitan area compared to the non-metropolitan area. The traditional dimension of activity while eating (eating only) no longer showed a significant pattern after being adjusted to sociodemographic factors.

The tendency to cook frequently was found among older age groups (36–45 and >45 years old) with aOR 1.444; 1.031–2.024 and 2.596; 1.850–3.642, also among Jawa-Madura ethnicity (2.016; 1.376–2.953). The highest income (T3) group (0.512; 0.388–0.675), subjects with high education (0.614; 0.406–0.927), and all-Sumatera ethnicity (0.647; 0.427–0.981) were less likely to cook frequently. Millennials usually prefer prepared foods and have more disposable income so they cook less

and consume more food away from home (Saksena et al., 2018). In metropolitan areas, numerous food retail retailers enable higher consumption of food prepared away from home (Stanton, 2015).

The 26–35 years and 36–45 years old age groups had higher odds to be eating away from home frequently (3.754; 1.936–7.279 and 2.336; 1.230–4.435, respectively). In contrast, non-workers (0.315; 0.153–0.648), housewives (0.199; 0.088–0.450), and subjects living in small households (0.310; 0.123–0.782) were less likely to eat away from home frequently. The increase in eating away from home might be related to adaptation to working life. In this study, subjects aged 26–45 years, who are mainly workers, tended to eat away from home frequently. Culture and social media can also influence eating out practice. In Indonesia, eating out with family and friends has almost become a culture as numerous dining options in urban areas, from hawker street foods to fancy restaurants, are available anywhere (Anyanwu et al., 2022) Through food vlogs and social media, information spreads among populous Indonesian netizens and influences food purchasing behavior (Vita et al., 2021).

No significant differences were found in the eating companion dimension between metropolization levels. The possible explanation might be that the increase in individualization was happening while socialization of eating prevailed, both in metropolized and less metropolized areas. This phenomenon was also found in Malaysia (Poulain et al., 2014) which can be explained by the similar characteristics between both countries, such as multiethnicity, rising economy, and rapid modernization (Poulain et al., 2020).

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Table 3. Differences in Eating Behavior in a Day after Adjusted with Sociodemographic Factors

Variable	Number of Eating Events		Food Preparation		Eating Location		Eating Companion		Activity while Eating	
	>5 eating events	OR (95% CI)	Cooking Frequently	OR (95% CI)	Eating at Home Frequently	OR (95% CI)	Eating Alone Frequently	OR (95% CI)	Eating Only Frequently	Eating while Doing Something Else Frequently
Metropolization Level (Ref. Non metropolitan)		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
	Semi metropolitan	1.572 (0.937-2.636)	0.454 (0.310-0.664)**	1.650 (1.032-2.640)*	0.650 (0.388-1.089)	1.640 (0.735-3.657)	0.697 (0.464-1.047)	0.933 (0.641-1.357)	1.292 (0.905-1.845)	
	Secondary metropolitan	2.303 (1.439-3.686)**	0.452 (0.311-0.658)**	1.917 (1.185-3.102)*	0.505 (0.312-0.815)*	2.206 (1.109-4.386)*	1.105 (0.767-1.590)	0.979 (0.676-1.420)	1.116 (0.801-1.554)	
	Primary metropolitan	1.932 (1.062-3.517)*	0.173 (0.105-0.286)**	7.428 (4.119-13.397)**	0.382 (0.206-0.708)*	4.706 (1.959-11.302)*	0.785 (0.483-1.276)	0.641 (0.395-1.042)	1.880 (1.212-2.914)*	
Age (Ref.18-25y ref)	26-35y	-	0.989 (0.726-1.348)	0.932 (0.639-1.359)	0.427 (0.273-0.668)**	3.754 (1.936-7.279)**	-	1.122 (0.782-1.609)	0.879 (0.637-1.213)	
	36-45y	-	1.444 (1.031-2.024)*	0.571 (0.373-0.873)*	0.637 (0.421-0.963)*	2.336 (1.230-4.435)*	-	1.521 (1.041-2.222)*	0.614 (0.435-0.866)*	
	>45y	-	2.596 (1.850-3.642)**	0.388 (0.255-0.590)**	0.820 (0.519-1.295)	1.911 (0.949-3.848)	-	1.984 (1.386-2.840)**	0.482 (0.349-0.666)**	
Sex (Ref. Male)	Female	-	-	-	0.790 (0.561-1.114)	1.047 (0.649-1.687)	1.591 (1.206-2.100)**	1.672 (1.255-2.229)**	1.601 (1.232-2.081)**	
Ethnicity (Ref. Sunda)	Betawi	0.957 (0.501-1.827)	0.922 (0.509-1.671)	1.072 (0.596-1.928)	0.906 (0.464-1.769)	1.483 (0.584-3.765)	1.966 (1.148-3.364)*	1.427 (0.775-2.626)	0.835 (0.489-1.427)	
	Jawa-Madura	0.615 (0.390-0.972)*	2.016 (1.376-2.953)**	0.539 (0.334-0.868)*	1.411 (0.852-2.335)	0.930 (0.425-2.034)	2.271 (1.584-3.254)**	2.122 (1.439-3.131)**	0.595 (0.418-0.849)*	
	Bali	0.629 (0.369-1.073)	0.680 (0.444-1.043)	1.635 (0.977-2.736)	1.213 (0.693-2.123)	1.450 (0.617-3.407)	7.144 (4.533-11.260)**	2.523 (1.618-3.934)**	0.550 (0.373-0.810)*	
	All Sumatra	1.291 (0.811-2.057)	0.647 (0.427-0.981)*	1.311 (0.798-2.153)	0.758 (0.457-1.255)	2.124 (0.979-4.608)	1.783 (1.179-2.696)*	0.829 (0.523-1.315)	1.224 (0.823-1.820)	
Income (Ref. T1)	Sulawesi	0.354 (0.192-0.652)**	1.445 (0.936-2.232)	1.163 (0.686-1.971)	1.372 (0.773-2.434)	1.767 (0.770-4.056)	0.653 (0.428-0.997)*	1.797 (1.151-2.807)*	1.042 (0.693-1.566)	
	T2	-	0.734 (0.523-1.031)	1.544 (1.012-2.357)*	-	-	1.402 (0.987-1.990)	-	-	
	T3	-	0.512 (0.388-0.675)**	1.553 (1.100-2.194)*	-	-	1.342 (1.014-1.775)*	-	-	

Education (Ref. Low)	Middle	-	0.788 (0.581-1.071)	1.035 (0.697-1.538)	0.601 (0.382-0.946)*	-	-	0.772 (0.572-1.041)	-
	High	-	0.614 (0.406-0.927)*	1.550 (0.950-2.529)	0.427 (0.245-0.742)*	-	-	0.678 (0.447-1.029)	-
Occupation (Ref. Workers)	Non-workers	-	-	0.943 (0.629-1.416)	2.535 (1.573-4.086)**	0.315 (0.153-0.648)*	1.295 (0.913-1.837)	0.818 (0.553-1.209)	1.551 (1.091-2.206)*
	Housewives	-	-	0.709 (0.512-0.982)*	3.080 (1.883-5.037)**	0.199 (0.088-0.450)**	0.833 (0.607-1.143)	1.101 (0.787-1.541)	1.079 (0.796-1.462)
Family size (Ref. Single-person HH)	Small HH	-	-	-	1.789 (0.839-3.814)	0.310 (0.123-0.782)*	-	-	-
	Large HH	-	-	-	1.194 (0.565-2.523)	0.468 (0.190-1.154)	-	-	-

* p<0.05
** p<0.001
- Not adjusted for the sociodemographic factor

In modernized society, formality was reduced as the social constraints grew more relaxed (Wouters, 2009). The findings on activity while eating in this study might indicate that the informalization process was happening. Higher use of social media and the internet in metropolitan areas (Koiranen et al., 2020) might influence the activity done while eating.

This study showed how metropolization might shift the eating behavior of adults in Indonesia into a more modernized pattern. Modern eating was related to an increased risk of obesity and NCDs since how people eat might influence what people eat. Higher total energy intake and later night-time eating were found to be related to the “grazing” pattern (Kahleova et al., 2017). Eating location was found to influence eating patterns and behaviors and resulted in diet quality. Leisure places, food outlets, and eating on-the-go may influence the type of food consumed as people are more exposed to non-core food in such places (Ziauddeen et al., 2017). Social influence shifts food intake, whether the amount or type of food consumed (Higgs, 2015). Stimuli from other activities done while eating were known to influence eating, resulting in higher food intake (Wagnild & Pollard, 2021).

Eating behavior in this study was defined using five dimensions that encompass the temporal aspect, preparation, spatial aspect, social aspect, and informalization. These combinations might provide a better understanding of eating behavior and become the strength of this study. However, this study did not differentiate the type of eating event (meal or in-between meal). It might be the limitation of this study since identifying the type of each eating event might result in more comprehensive data as different eating events might have different characteristics. A separate analysis of the variables used to define eating behavior might also reduce the understanding since each variable might interact with one another. Further studies should explore the possibility of these interactions.

City size can be determined using several indicators, such as economic indicators (e.g., GRDP, unemployment rates), social indicators (e.g., crime rates, social interaction), and demographic indicators (e.g., population size). Based on the consensus, the demographic indicator is used to define the city size

(Adisasmita, 2013). This study used a new approach by combining economic and demographic indicators assuming their influence on the food and social environment in the metropolitan area. However, since it was not a standardized approach, further cross-disciplinary research might still be needed to develop the standard for defining metropolization level as misclassification potential exists.

CONCLUSION

This study showed that people in more metropolized areas have more tendency to have more modernized eating behavior, i.e. having >5 eating events/day, not cooking frequently, and eating away from home frequently. More areas will grow into larger cities and metropolises as Indonesia develops. Urban development should align with sustainable development goals (SDGs) and New Urban Agenda goals. Improving access to healthy food, providing a healthy food environment, and work environment to enable people to have better eating behavior are necessary to build a healthy city. Building supporting facilities (e.g., efficient & sufficient mass transport system to reduce traffic jams and commuting time) and enforcing a conducive working environment (e.g., working hours, reasonable workload) could improve quality of life that people have more time to eat proper meals, preparing home-cooked food, and eat with the family. Good urban planning might reduce barriers and enable people in metropolized areas to eat better.

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THE RELATIONSHIP BETWEEN FOOD CONSUMPTION, INCOME, AND FOOD EXPENDITURE WITH HOUSEHOLD FOOD SECURITY IN CARINGIN DISTRICT, BOGOR

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ABSTRACT

Hunger is a condition of food insecurity. One of the efforts that can be made to reduce the incidence of food insecurity is to maintain food security, especially in households. This research aimed to analyze the relationship between food consumption and income and food expenditure with household food security in Caringin District, Bogor Regency. This is an analytic observational study with a cross-sectional design with a random sample of 60 households in Caringin district, Bogor regency. Assessment of household food availability based on a food availability questionnaire consisted of several types of food groups. Income and expenditure were measured by assessing household income. The condition of household food diversity measured using the HDDS questionnaire. The chi-square test was used to analyze the relationship between consumption behavior, income, household expenditure, and household food security. There was a relationship between income ($p < 0.001$) and expenditure ($p < 0.001$) with household food security, while consumption behavior is not ($p > 0.05$). It is concluded that there was a relationship between income and expenditure on food security, but behavior did not show an insignificant relationship. There needs to be an in-depth analysis to find other factors influencing food security apart from the variables examined in this study.

Keywords: consumption behavior, household, income, food security, expenditure

INTRODUCTION

Poor diet is a major cause of death and morbidity worldwide, exceeding the burden caused by many significant global health challenges, one of which is the resulting global malnutrition crisis, including hunger and malnutrition. Hunger is caused by insecurity that occurs in the household (Dhamija et al., 2022). Food insecurity is responsible for poorer health (Alaimo et al., 2020). Hunger is identified with chronic malnutrition, a person's inability to obtain sufficient food to meet minimum daily dietary energy requirements for one year (Webb et al., 2018).

Famine is again looming throughout the world due to the Covid-19 pandemic. It is projected that between 720 and 811 million people in the world will face hunger in 2020, or an estimated increase in the number of more than half of the world's malnourished population found in Asia (418 million) and more than a third in Africa (282 million). Compared with 2019, approximately 46 million more people in Africa, 57 million more in Asia, and approximately 14 million more in

Latin America and the Caribbean were affected by hunger in 2020. The global prevalence of moderate or severe food insecurity (measured by the Experience of Food Insecurity Scale) has slowly increased since 2014; one in three people in the world, or around 2.37 billion people, did not have access to sufficient food in 2020 or the equivalent of an increase of almost 320 million people in just one year. One form of business that can be done is to increase food security to end hunger, food insecurity, and malnutrition (FAO, 2021).

Not everyone has the ease of obtaining the food they need; this leads to hunger and malnutrition on a large scale worldwide. Part of the world's population now experiences chronic food shortages and cannot obtain enough food to meet energy needs. Millions of children under five years old (toddlers) suffer from chronic or acute malnutrition during food shortages, seasons of hunger, and social unrest (Kementerian PPN/ Bappenas, 2023).

The aim of building food security is to ensure the availability and consumption of sufficient,

safe, quality, nutritious, and balanced food at the household, regional, and national levels at all times and evenly and is affordable and does not conflict with the religion, beliefs, and culture of the community, to be able to live a healthy, active and productive life in a sustainable manner (UUD RI, 2012).

The direct causes of nutritional problems are food intake and disease. According to UNICEF, food intake is determined by food availability. Food availability is related to the physical presence of food in sufficient quantities. This availability includes aspects of quantity and quality. Several studies show a positive relationship between food diversity and quality, micronutrient intake for children under five, and household food security (Antwi et al., 2022; Jun et al., 2019). Apart from that, research conducted by (Prasetyaningtyas, 2017) Found a relationship between food availability and the food diversity of farmworker households (Prasetyaningtyas & Nindya, 2018).

Availability of food and sufficient food consumption are factors in obtaining a variety of safe, quality, and nutritious food. This depends on the level of poverty in the household. Food security will have a significant impact on reducing poverty. Bogor Regency is one of the districts that has experienced an increase in population below the poverty line, amounting to 7.69% of the population below the poverty line. Researchers are interested in studying one of the sub-districts, namely Caringin, because, based on data from Caringin Sub-district, there are still quite a lot of households that fall into the underprivileged group (Badan Pusat Statistik Kabupaten Bogor, 2021). This research aims to analyse food availability, income, expenditure, and household food diversity in Caringin District, Bogor Regency.

METHODS

This research is an analytical observational study with a cross-sectional design. The population in this study was all households in Caringin Village, Caringin District, Bogor Regency. The samples were taken using the random sampling technique. The minimum sample required using the estimation formula was 60 households in the selected sub-districts. The basis for calculating the sample based on food security index data in Bogor Regency in

2020 with a random household sampling technique. Data collection in this research carried out by interviews using a structured questionnaire related to food availability and expenditure and a food consumption diversity questionnaire.

Assessment of household food availability based on a food availability questionnaire, which consisted of several types of food groups (animal source food, vegetable source food, fruit, milk/dairy products, and vegetables) that are available or not available. Household food availability categorized into two compared with normative consumption per individual (300 grams per day), namely insufficient if food availability <300 grams/day and sufficient if food availability (Santi & Andrias, 2015).

Income and expenses are measured by assessing household income consisting of the number of household members who have income; the amount of income then asked about the frequency, daily, weekly, or monthly (Widyareni, 2011). Total income calculated for one month from the frequency of household income. The assessment of household expenditure grouped into two, namely food and non-food expenditure, then the amount of expenditure and frequency of expenditure measured and then totaled into expenditure in one month. The proportion of food expenditure calculated by the percentage of food expenditure compared to total expenditure. The proportion of food expenditure classified into 2, namely < 60% and $\geq 60\%$ (Widyareni, 2011).

The condition of household food diversity measured using the Household Dietary Diversity Score (HDDS) questionnaire. The HDDS score calculated by writing the number 1 for the type or group of food consumed and 0 if it is not consumed. Then, add up all the scores from all food groups. The total results range from 0 to 12. The HDDS score grouped into 3 criteria: a low score of less than 3, a medium score of 4-5, and a high score of more than 6. Next, the average HDDS score for the household calculated (Swindale & Bilinsky, 2006).

Household food security status measured using the United States Household Food Security Survey Module (US-HFSSM) form, which then classified as food secure if the value between 0-2, food insecure without hunger between 3-7, food insecure with degrees moderate hunger between

8-12, and food insecurity was severe hunger if the value between 13-18 (Bickel et al., 2000).

The statistical analysis used to determine the relationship between consumption behavior, income, and household expenditure with household food security is the Chi-Square test with $p < 0.05$.

RESULTS AND DISCUSSIONS

General characteristics of respondents showed that most respondents were aged 18-40 years (72.2), and the rest were aged 41-65 years (27.8%). This shows that most respondents are of productive age. The highest level of education of respondents was high school (61.1%), while the least was elementary school (2.8%). Most respondents' occupations were not working or acting as a housewife (54.2%), and 15.3% of respondents had jobs other than being self-employed and private employees.

Table 1. Characteristics of Respondents

Characteristics	n (%)
Age	
18 – 40 y.o	52 (72.2)
41 – 65 y.o	20 (27.8)
Educational background	
Elementary school	2 (2.8)
Junior high school	8 (11.1)
Senior high school	44 (61.1)
College	18 (25.0)
Occupation	
Housewife	39 (54.2)
Self-employed	9 (12.5)
Entrepreneur	6 (8.3)
Private sector employee	7 (9.7)
Other	11 (15.3)

Table 2 shows that almost all respondents eat various types of food. However, the type of food

that respondents consumed the lowest was fish; only 84.7% of respondents consumed it. This is supported by previous research, which shows that most of the research conducted in rural Indonesia from 2000-2015 showed a decline in food groups such as fish. This based on previous research that low consumption of fish is influenced by several factors, one of which is household production (Mehraban & Ickowitz, 2021).

Table 2. Distribution of Food Diversity Consumed According to Respondents

Type of Food	Consumption Pattern n (%)	
	Yes	No
Wheat	72 (100)	0 (0.0)
Vegetables	72 (100)	0 (0.0)
Tubers	72 (100)	0 (0.0)
Fruit	72 (100)	0 (0.0)
Meat	72 (100)	0 (0.0)
Eggs	72 (100)	0 (0.0)
Fish	61 (84.7)	11 (15.3)
Nuts	70 (97.2)	2 (2.8)
Dairy products	72 (100)	0 (0.0)
Foods made from oil, fat, or butter	72 (100)	0 (0.0)
Sugar/honey	72 (100)	0 (0.0)
Other foods	71 (98.6)	1 (1.4)

Table 3 shows that 73.6% of households had insufficient food availability (individual normative consumption in the household <300 g). Bivariate analysis was carried out to measure the relationship between normative cereal consumption behavior and food security status. A correlation test using Spearman with $\alpha = 0.05$ obtained a $p = 0.14$, meaning there is no significant relationship between normative cereal consumption behavior and the status of food security in society.

Table 3. Relationship between Normative Cereal Consumption and Food Security Status

Normative Cereal Consumption	Food Security Status						Total	p- value	
	Food insecure, moderate degree of hunger		Food insecure, without hunger		Food security				
	n	%	n	%	n	%	n	%	
< 300 g	7	9.7	17	23.6	29	40.3	53	73.6	0.14
≥ 300 g	1	1.4	4	5.6	14	19.4	19	26.4	
Total							72	100	

Table 4. Relationship between Monthly Food Expenditure and Food Security Status

Monthly Food Expenditures	Food Security Status						Total		P value
	Food insecure, moderate degree of hunger		Food insecure without hunger		Food security				
	n	%	n	%	n	%	n	%	
≥ 60 %	0	0.0	0	0.0	16	22,2	16	22.2	<0.001
< 60%	8	11.1	21	29.2	27	37.5	56	77.8	
Total							72	100	

These results align with research conducted by Santi dan Andrias (2015) in Lumajang, which resulted in an analysis of no relationship between food stock availability as measured by household cereal consumption and food security status. Other research shows different results indicating a relationship between normative household food consumption and food security status (Hapsari & Rudiarto, 2017). Food availability generally influences food security status because it determines the degree of hunger and household consumption. Food availability is influenced by other factors, such as the number of family members, age, and food allocation in the household (Drammeh et al., 2019). In Caringin District, this insignificant relationship could occur due to variations in age within households and different allocations in terms of food consumption. So, household members ignore how much food they should consume.

Most households had food expenditure <60% of their monthly expenditure (Table 4). Correlation analysis using Spearman’s rank obtained p value <0.001, meaning there was a significant relationship between monthly food expenditure and household food security status in Caringin District. This aligns with research Sutyawan, Khomsan, dan Sukandar (2019), that a real negative relationship exists between the food security index and the proportion of household food expenditure. Higher food expenditure compared to other needs makes households more vulnerable to food insecurity.

This condition usually occurs in low-income families (Kh’ng et al., 2022). Households with low income will focus on buying food without paying attention to its nutritional content. Finally, the proportion of food expenditure will be greater than other expenditures. This is different from households that have higher incomes. High-income households will consume varied and nutritious foods (Manyullei & Arundhana, 2021).

After grouping respondents’ income based on the District Minimum Wage, it was found that 69.4% of respondents had an income over the district minimum wage. The remaining 30.6% had incomes below the district minimum wage, and it was known that some of them worked odd jobs with an uncertain income. The results of the Spearman rank correlations test showed p value<0.001, which means there was a significant relationship between monthly income and food security status, meaning that the lower the respondent’s income, the more food insecure they are (Table 5).

In Ethiopia, households with low incomes are 5 times more likely to experience food insecurity than households with high incomes (Derso et al., 2021). Food insecurity due to low income also occurred in England in 2004-2016, with an increase of 18.1% of households experiencing food insecurity. It is also associated with poor health and disability (Loopstra et al., 2019). The unique thing that can be found in this research is that several households with low incomes can still avoid food

Table 5. Relationship between Monthly Income and Food Security Status

Monthly income	Food security status						Total		P value
	Food insecure, moderate degree of hunger		Food insecure without hunger		Food security				
	n	%	n	%	n	%	n	%	
≤ Rp 2.975.000 (Low)	7	31.8	10	45.5	5	22.7	22	30.6	<0.001
> Rp 2.975.000 (High)	1	2.0	11	22.0	38	76.0	50	69.4	
Total							72	100	

insecurity (5%). Although low income is a fairly strong factor in predicting household food security status, this parameter cannot be the only reason behind food insecurity (Pool & Dooris, 2022). The results of research in Bogor are not different from those in other places where low income is associated with food insecurity. As shown in Table 5, the majority of respondents have incomes above IDR 2,975,000, perhaps because the location of the Caringin sub-district borders the Sukabumi district and is an industrial location.

CONCLUSION

This research concludes that many households still have food insecurity status in Caringin Village, Caringin District, Bogor Regency. The analysis results show that food security status is caused by the proportion of monthly food expenditure and monthly income. The way to manage monthly expenses and income is that there needs to be a role for the housewife in the household in arranging a food menu that is more varied and highly nutritious. Therefore, it is necessary to increase mothers' knowledge as one of the dimensions of food security in the aspect of affordability. Analysis shows there is no relationship between food availability and food security. The weakness of this research is that respondents' participation is still relatively small. Apart from that, there is a need for in-depth analysis to find out other factors that influence food security apart from the variables examined in this research. In the future, this research will encourage households to increase their food security by paying more attention to local food. In addition, strengthening human resources by empowering small-scale food businesses, which is the dominant feature of the Indonesian agricultural economy, requires aligning or integrating small-scale food business activities into the food supply chain.

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THE HOUSEHOLD FOOD SECURITY AND STUNTING OF UNDER-FIVE CHILDREN IN INDONESIA: A SYSTEMATIC REVIEW

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ABSTRACT

Inadequate access to food at home contributes to growth retardation in childhood. Under-five children who suffer from stunting have problems in their growth and development owing to a lack of nutrients over an extended period of time. This study aimed to analyze the relationship between household food security and stunting prevalence. This study uses a systematic review using Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA). Seven electronic databases are used: PubMed, ProQuest, MDPI, Science Direct, Scopus, Google Scholar, and Portal Garuda. This research was based on studies conducted in Indonesia and published between 2013 and 2023. For the final result, sixteen articles were included. Household food security has a positive correlation with the stunting of under-five children in Indonesia. Moreover, households with food insecurity had a greater risk of stunting. A household is said to be food-secure when access to food is fulfilled by all family members. From this systematic review, the government is expected to formulate policies and strategies to improve household food security and the nutritional status of children under the age of five. To accomplish these efforts, coordination between sectors is required to accelerate and strengthen the implementation of these policies and strategies.

Keywords: children, food insecurity, malnutrition, stunting

INTRODUCTION

A substantial problem faced by the world today is poverty. This occurred due to economic conditions affected by the COVID-19 pandemic, where Indonesia's status changed from a high-middle death country to a low-middle death country starting in July 2021 (World Bank 2022). The COVID-19 pandemic also increased the poverty rate, where the poverty percentage in Indonesia was 9.71% in September 2021, and there was a decrease in March 2023, namely 9.36% or the equivalent of 25.90 million people (Statistics Indonesia 2023). Poverty is the main factor causing food insecurity. Low-income families often do not have enough money to buy sufficient, nutritious, and affordable food. This may be a sign of an inability to attain food security. Food security is defined by Law No. 18 of 2012 as a location where all residents have constant and unfettered access, throughout their life course, to sufficient, safe, diverse, nutritious, equitable, and affordable food, without prejudice to any person or group's faith, practice, or cultural background, in order to achieve and maintain an optimal level of physical and mental health and social and economic well-being (Ariani & Suradisastra, 2013). Food security is

also one of the many objectives in the Sustainable Development Goals stated in the second point (zero hunger). Meeting one's nutritional needs is at the heart of the concept of food security, which encompasses not only food supply at the regional level but also food availability and intake at the regional, household, and individual levels.

Under-five stunting is significantly linked to food poverty at home. Stunting can be caused by inadequate nutritional intake, that can last for a relatively long time. Inadequate nutritional intake is a condition of household food insecurity, where its availability and access are less (lacking), so food or nutritional intake is not fulfilled. If a household experiences food insecurity for a certain period, this can result in a lack of nutritional intake and impact an individual's nutritional status (Arlius et al., 2017).

Nutritional problems in toddlers have become a major issue in Indonesia. Data from the Indonesian Nutritional Status Survey (2021) reported that the prevalence of toddlers being underweight was 17%, wasting was 7.1%, and stunting was 24.4%. The figure shows that toddlers' nutritional problems still become a significant health problem that can cause permanent damage

to children's physical and mental health and affect their health and productivity as adults. Malnutrition in the first 1000 days of life can cause poor cognitive and physical health in toddlers because this period is essential for brain development and linear growth (De Onis et al., 2013).

The 1000 days of life is an early period of life, starting from the fetus being formed in the womb (270 days) to the first two years of life (730 days), popularly termed the Golden Age (Levinson et al., 2013). This is a crucial period because children's developmental conditions are progressing rapidly, which is also very vulnerable since this period will impact the quality of future generations. Within this period, nutritional intake must be considered starting from the time of a couple before their marriage, expectant mother, during pregnancy (fetus), until the children phase, because if the nutritional intake is low, this lack will cause disorders in the growth and development of the toddler. The 1000 Days of Life movement aims to improve the commitment of stakeholders to accelerate the accomplishment of the nutrition improvement target and strengthen the implementation of the nutrition program concept in direct and indirect ways by conducting two types of integrated interventions that cannot work alone and require collaboration between the government and the community; in the form of a specific nutrition intervention and a sensitive nutrition intervention (Levinson et al., 2013). Food security is an important factor that needs to be considered in efforts to reduce the prevalence of stunting. Effective food security interventions can be an important strategy to achieve the 2030 Sustainable Development Goals (SDGs), namely ending hunger, achieving food security and improving nutrition. From the description above, this systematic review was created to look at the relationship between food security and stunting in Indonesia. It can significantly contribute to efforts to reduce the prevalence of stunting in Indonesia.

METHODS

Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) were used as the framework for this systematic review (Moher et al., 2009). Articles were searched in seven databases: PubMed, ProQuest, MDPI, Science Direct, Scopus, Google Scholar, and

Garuda Portal. The selected articles were published between 2013 to 2023 in both English and Bahasa. The search for articles was carried out from May to July 2023, with four keywords used in the search process by the boolean operators "AND" and "OR" as follows:

- a. ("household food security" OR "food security") AND ("stunting" OR "stunt") AND ("Indonesia" OR "Indonesian")
- b. ("household food insecurity" OR "food insecurity") AND ("stunting" OR "stunt") AND ("Indonesia" OR "Indonesian")
- c. ("ketahanan pangan rumah tangga" OR "ketahanan pangan") AND ("stunting") AND ("Indonesia")
- d. ("kerawanan pangan rumah tangga" OR "kerawanan pangan") AND ("stunting") AND ("Indonesia")

The inclusion criteria were as follows: (1) unique papers on the subject of this study; (2) the research studies chosen were carried out in Indonesia; (3) children under the age of five were the primary study participants; (4) observational research designs (cross-sectional, case-control, and cohort) were used for this investigation; (5) the research included a means of gauging food security at the household level; (6) the quality of the articles (indexed in SINTA and Scopus); and (7) articles that were fully accessible. The exclusion criteria were study design other than observational design (cross-sectional, case-control, and cohort) and unpublished opinions, books, and studies or abstracts.

All articles identified by the search engines were then exported to the Mendeley application to determine whether any similar articles were later deleted. Writers read the titles and abstracts of all articles. The researchers performed the last round of screening by reading all publications that met the inclusion and exclusion criteria. The authors conducted and evaluated all data extraction and research evaluation on their own. Articles were selected based on their relevance to the research question, and data were extracted into a table that included the author, publication year, sample size, sample age, study goals, method of assessing household food security, and the correlation between household food security and stunting in children under the age of five.

RESULTS AND DISCUSSIONS

From the search results of the seven electronic databases, 6.954 articles were initially obtained according to predetermined keywords. After deleting the same articles, 4.002 articles were obtained and reviewed to determine the suitability aspect of the title and abstract related to the aim of this study. Finally, 131 articles were selected for this study. The next stage of this study was to review the articles by reading their contents for further study according to the inclusion and exclusion criteria previously set in this study. This, resulted in 16 articles that could be analyzed in this study. The study stages of the search, selection process, and appropriate articles based on the inclusion and exclusion criteria are presented in Figure 1.

Table 1 displays the broad features of the studies considered in this systematic review. There were sixteen in Indonesia, seven of which were found in East Java and five in Central Java. The remaining studies (consisting of one study in each of the following regions) were from West Java, Central Sulawesi, Jambi, and North Sumatra. The subjects in this study were children under five years old (0-59 months), with sizes ranging from 96 to 736 households and 59 to 300 children. The majority of the study designs in these studies were cross-sectional, and four studies used case control methods. Measures of household food security can be conducted in a various ways. The Household Food Insecurity Access Scale (HFIAS) was used in seven investigations. The HFIAS method can be used to measure food security because it is an easy and practical method (Ashari et al., 2019). Meanwhile, five studies use the United States-Household Food Security Survey Module (US-HFSSM), as supported by a statement of US-HFSSM, which can be used to measure household food security (Ellison et al., 2021). Three studies used questionnaires prepared by their respective research teams, and one research study used the Household Dietary Diversity Score (HDDS). The HHDS score is the best proxy for assessing household food security (Hussein et al., 2018; Ngema et al., 2018).

According to the definition provided by Arlius et al. (2017), when everyone in a society is able to eat a healthy, balanced diet, we have achieved food security, is free from contamination, is culturally

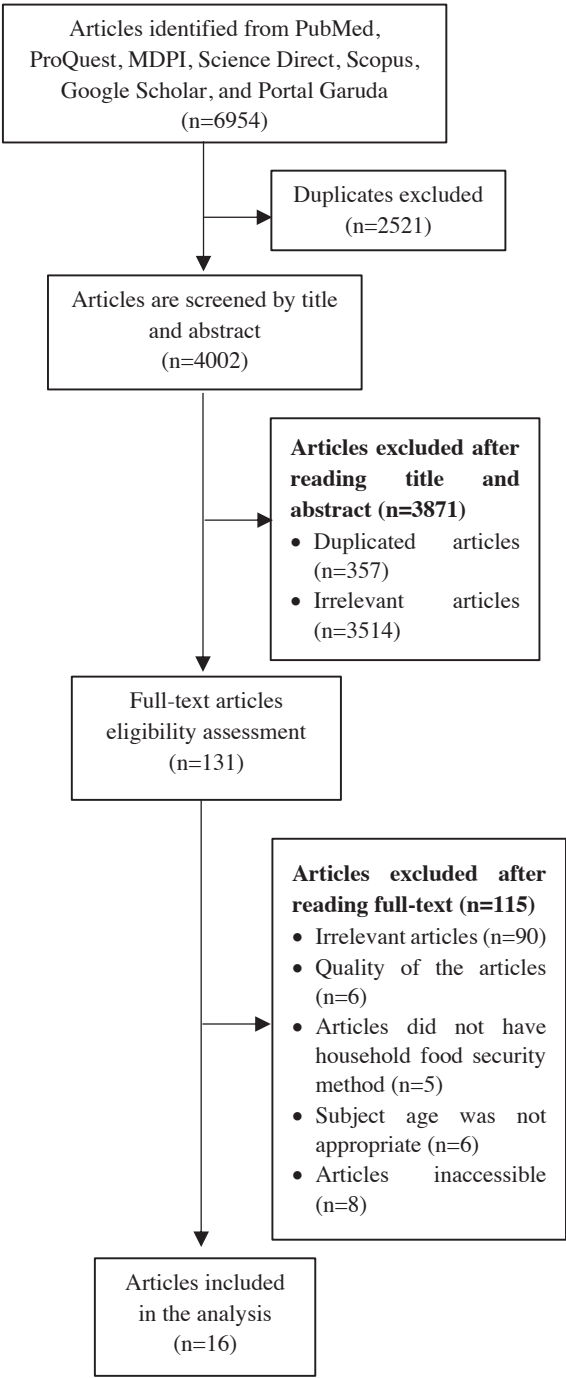


Figure 1. Flowchart of Articles Selection Process by PRISMA

acceptable, is fairly distributed, is affordable, and promotes good health and well-being over the long term. Home food security is essential for adequate food intake, leading to excellent nutritional status (Yang et al., 2019). If a household cannot meet adequate food necessities, it will affect the nutritional intake of children under five years of

age, eventually leading to stunting (Yanti et al., 2023). Stunting is a disease caused by chronic malnutrition (Sadiq et al., 2023). This growth disorder able to cause permanent damage (Adelia et al., 2018).

Children under the age of five who were not receiving enough to eat at home were not more likely to be stunted, according to two of the 16 articles analyzed. No correlation was found between household food security and stunting, according to a study conducted in Gunungkidul (Delima & Septriana, 2019) and other research in Sukoharjo showed that food availability is not related to toddler growth (Aryati et al., 2018). However, 14 studies suggest that a lack of family food security is linked to stunting in children younger than five.

Meanwhile, research conducted in Surabaya has reported that the HFIAS method can predict SCWOT in Indonesia (Mahmudiono et al., 2018). Several other studies have used the HFIAS methodology and have shown a correlation between low levels of food security at home and the prevalence of stunting. Toddlers in food insecure homes had a whopping 10.9 fold higher risk of stunting, according to studies conducted in Bogor (OR= 10.9 95 % CI=1.8-67.3) (Utami & KP, 2015). This finding is also in line with research conducted in Gicumbi, Rwanda, which found that children living in food-insecure homes had a 2.47-fold higher risk than stunting as toddlers (AOR= 2.47; CI= 1.77, 3.46) (Kingsley et al., 2018).

In addition, studies conducted in several parts of East Java have revealed regional variations in food insecurity among households. Food insecurity and malnutrition are more common among households in coastal and limestone regions. The highest food insecurity condition was found in coastal areas (7.2%) and in the karst limestone area (5.3%), while the highest stunting prevalence was found in coastal areas (11.6%). Therefore, according to Sumarmi et al. (2018), geographic factors affect household food security and toddler nutrition. These findings corroborate those of a Brazilian study that found that the majority of people living in coastal regions are economically dependent on fishing and hence more susceptible to the effects of climate change. In addition, a similar study was found in Tapanuli with a

significant relationship between food security and the nutritional status of children under five years old. This study also revealed that the bulk of the local workforce is comprised of fishermen, and that toddlers living in food-insecure homes are 6.3 times more likely to be underweight ($p= 0.003$; OR= 6.30) (Masthalina et al., 2021).

In agreement with this discussion, numerous additional studies (Raharja et al., 2019; Asparian et al., 2020; Islamiah et al., 2022) have shown that a high rate of stunting in children younger than five years is correlated with low levels of food security in the home. However, other variables had a substantial association with the prevalence of stunting aside from household food security, including mother's education, household food expenditure, birth length, history of exclusive breastfeeding, family economic position, and socio-demographics. According to research conducted in Jambi, household food security (OR= 4.722; 95% CI= 1.599-13.941) is the factor most associated with stunting in children under five, apart from the mother's education level which also related to the stunting incidence ($p<0.05$). After accounting for differences in household income and parenting styles, toddlers born to mothers with lower education levels had a 2.554 times higher risk of stunting than those born to mothers with higher education (Asparian et al., 2020). Moreover, parents' economic status ($p=0.002$; OR=3.182) and family food security ($p=0.007$; OR=3.164) were risk factors for stunting in toddlers (Raharja et al., 2019), as reported by researchers in Pasuruan and Semarang, who found that household food expenditure, birth length, history of exclusive breastfeeding, and socio-demographics are significantly related to stunting incidence (Sudargo & Armawi, 2019; Islamiah et al., 2022). These results also align with research conducted in Rwanda, which that reported the determinants of stunting, including gender, toddler age, mother's age, and mother's education (Nshimiyiryo et al., 2019). Research conducted in Indonesia also revealed that the determinant factors that influence stunting incidence are the sex of the children, the duration of breastfeeding for more than 24 months, the children's age, the period of Early Breastfeeding Initiation (*Inisiasi Menyusui Dini*), growth monitoring, mother's age, mother's

Table 1. Summary of selected studies

No.	Study references	Study design	Charaistic of subject	Location	Methods of HFS	Study purpose	Result
1.	Sumarmi et al. 2018	Cross-sectional	<ul style="list-style-type: none">• 96 households for each region with 768 households, yet only 736 were included in the inclusion criteria.• Children under five years old	East Java Province <ul style="list-style-type: none">• Coastal area: Pasuruan and Lamongan• Karst/limestone area: Gresik and Pamekasan• Agriculture area: Ngawi and Banyuwangi• City area: Madiun and Blitar	US-HFSSM	<p>To analyze the relationship between household food insecurity and malnutrition in under five children from different geographical areas (coastal, limestone, agricultural, and urban areas) in East Java Province.</p> <ul style="list-style-type: none">• The prevalence of stunting is greatest (11.6%) in coastal areas, whereas the prevalence of wasting (6.2%) and underweight (8.9%) are highest in karst/limestone regions.• The highest prevalence of food insecurity is found in coastal areas (7.2%) and karst/limestone areas (5.3%).• Differences in household food insecurity and the nutritional health of children under age five are linked to locations. Children under the age of five were more likely to suffer from food insecurity and malnutrition in homes located near the seaside or in locations rich in limestone.	
2.	Muslihah et al. 2022	Cross sectional	<ul style="list-style-type: none">• 300 toddlers• 6-23 months	Madura, East Java	HFIAS	<p>To investigate the connection between household food security and the improper feeding of complementary/weaning foods to 6-23-month-old anemic and stunting toddlers.</p> <ul style="list-style-type: none">• Households experiencing food insecurity are found with a percentage of 65.3%.• The prevalence of stunted and anemic toddlers is higher in food insecurity households (36% and 48%) when compared to food secure households (31.7% and 43.3%).	
3.	Mahmudiono et al. 2018	Cross-sectional	<ul style="list-style-type: none">• 685 households• Children under five years old	Surabaya, East Java	HFIAS	<p>Researching the association between SCOWT (Stunted Children and Overweight/Obese Mother) and food insecurity in Indonesian households.</p> <ul style="list-style-type: none">• Stunting rates are not linked to food insecurity in the home.• Household food insecurity found is in the category of light food insecurity.	The household food insecurity analyzed through the HFIAS method can be a SCWOT predictor in Indonesia.
4.	Delima & Septriana 2019	Cross-sectional	<ul style="list-style-type: none">• 73 toddlers• 6-59 months	Gunungkidul, DI.Yogyakarta	HFIAS	<p>The goal of this research is to better understand the connections between parents' food insecurity and their children's risk of stunting.</p> <ul style="list-style-type: none">• Food security is significantly related to stunting status ($p<0.05$).• For a household with food secure status found with a percentage of 46.9%, a household with light food insecure status has a percentage of 28.1%, and households with moderate and heavy food insecure status have a percentage of 15.6% and 9.4% as taken from a population of survivors after four years of earthquake and tsunami's natural disaster.	
5.	Adhyanti et al. 2022	Cross-sectional	<ul style="list-style-type: none">• 96 households• 0-59 months	Palu, Central Sulawesi	HFIAS	<p>Examining the four-year-later food security and nutritional condition of tsunami and earthquake survivors' households.</p> <ul style="list-style-type: none">• Food security is significantly related to stunting status ($p<0.05$).• For a household with food secure status found with a percentage of 46.9%, a household with light food insecure status has a percentage of 28.1%, and households with moderate and heavy food insecure status have a percentage of 15.6% and 9.4% as taken from a population of survivors after four years of earthquake and tsunami's natural disaster.	

Table 1. Summary of selected studies (continue)

No.	Study references	Study design	Characteristic of subject	Location	Methods of HFS	Study purpose	Result
6.	Aryati et al. 2018	Cross-sectional	<ul style="list-style-type: none">130 toddlers12-24 months	Sukoharjo, Central Java	US-HFSSM	<p>The purpose of this study is to examine the impact of food insecurity on the development of children between the ages of 12 and 24 months old by looking at their intake of protein and zinc throughout the course of the 1000 Days of Life.</p>	<ul style="list-style-type: none">There is no relationship between poor household food security status and the growth and development period of toddlers/under two children ($p>0.05$).Intake of protein (OR=1.068; 95% CI=1.035-1.103), and zinc (OR=1.31; 95% CI=1.314-2.025) correlate to toddlers' growth and development period.
7.	Asparian et al. 2020	Cross-sectional	<ul style="list-style-type: none">98 toddlers24-59 months	Kerinci, Jambi	HDDES	<p>The objective of this study is to examine the causes of stunting in children aged 24 to 59 living in agricultural families.</p>	<p>The most related factor to stunting incidence in toddlers is household food security status (OR= 4.722; 95% CI=1.599-13.941).</p>
8.	Fadzila & Tertiaryus 2019	Case-control	<ul style="list-style-type: none">72 toddlers6-23 months	Nganjuk, East Java	US-HFSSM	<p>To compare the household food security of children who are stunted (6-23 months) with that of children who are not stunted.</p>	<p>There is a significant negative relationship between stunting and household food security status.</p>
9.	Islamiah et al. 2022	Cross sectional	<ul style="list-style-type: none">87 households6-59 months	Pasuruan, East Java	HFIAS	<p>The goal of this study is to examine the factors at play in the occurrence of stunting among toddlers, including family characteristics, child characteristics, and household food security.</p>	<p>There is a significant relationship between household expenditure, baby birth length, exclusive breastfeeding report, and household food security condition to stunting incidence in toddlers.</p>
10.	Masrin et al. 2014	Case-control	<ul style="list-style-type: none">126 toddlers6-23 months	Particular Region of Yogyakarta, Central Java	Questionnaire	<p>To investigate the connection between household food security and stunting in young children (6–23 months).</p>	<p>There is a substantial correlation between household food security and stunting prevalence (OR=2.62, 95% CI=0.97-7.12).</p>

Table 1. Summary of selected studies (continue)

No.	Study references	Study design	Characteristic of subject	Location	Methods of HFS	Study purpose	Result
11.	Masthalina et al. 2021	Cross-sectional	<ul style="list-style-type: none">• 59 toddlers• Under-five children	Tapanuli, North Sumatra	HFIAS	In coastal regions, toddlers' nutritional status is a major concern, therefore we need to examine the connection between food insecurity at home, nutritional sufficiency, and household food insecurity.	In coastal regions, there is a substantial correlation (p=0.003; 0.003 OR=6.30) between household food security and toddler malnutrition.
12.	Riski et al. 2019	Cross-sectional	<ul style="list-style-type: none">• 64 toddlers• 1-5 years	Surabaya, East Java	US-HFSSM	To analyze the household food security, illness, sanitation of the environment, toddlers' nutrition status	There is a significant relationship between household food security, illness, and sanitation of the environment and toddlers' nutritional status.
13.	Raharja et al. 2019	Case-control	<ul style="list-style-type: none">• 141 toddlers• 24-59 months	Bejiharjo village, Central Java	Questionnaire	To what extent do parents' socioeconomic status and food insecurity correlate with the frequency of toddler stunting is the focus of this research.	Toddler stunting becomes a risk factor when parents' socioeconomic position (p=0.002; OR=3.82) and food security (p=0.007; OR=3.164) are poor.
14.	Safitri & Nindya 2017	Cross-sectional	<ul style="list-style-type: none">• 68 toddlers• 13-48 months	Surabaya, East Java	US-HFSSM	To compare the prevalence of diarrhea and subsequent stunting in young children with the food security of their households.	<ul style="list-style-type: none">• There are 30.9% of stunted children, 61.8% of children were in food insecurity conditions, and 19.1% experienced diarrhea.• Household food security and stunting incidence show a significant relationship.
15.	Sudargo & Armawi 2019	Case control	<ul style="list-style-type: none">• 80 toddlers• 1-5 years	North Semarang, Central Java	Questionnaire	The goal of this study is to investigate the relationship between toddler stunting and the household's food security and other socioeconomic factors.	Household food security and socio-demographics have a significant relationship to stunting incidence.
16.	Utami and KP 2015	Cross-sectional	<ul style="list-style-type: none">• 216 toddlers• 6-23 months	Bogor, West Java	HFIAS	To examine how household food security affects the prevalence of stunting in young children.	Children under the age of two who are at risk for stunting have lower rates of household food security (OR= 10.9 95% CI=1.8-67.3)

education, mother's occupation, the birth length, and history of diarrhea (Permanasari et al., 2021; Herbawani et al., 2022).

Furthermore, research conducted in Nganjuk, Surabaya, Yogyakarta, Bogor, Surabaya, and Palu reported that a correlation exists between household food security and the stunting of children under the age of five (Masrin et al., 2016; Safitri & Nindya, 2017; Fadzila & Tertiyus, 2019; Riski et al., 2019; Adhyanti et al., 2022). Children in Ethiopia who experienced food insecurity were shown to have a 6.7 times risk of stunting than those who lived in food-secure families (AOR= 6.7; CI= 3.71-12.1) (Betebo et al., 2017). Other studies conducted in Kenya have reported a relationship between household food insecurity and stunting incidence (Mutisya et al., 2016). Children under the age of five in food-insecure homes had a greater frequency of stunting and anemia than toddlers in households without food insecurity, according to a study performed in Madura. These results show a significant association between food insecurity at home and the incidence of stunting and anemia. Reduced supplemental feeding due to food poverty at home has been linked to stunted development and anemia (Muslihah et al., 2022).

Families that struggle to put food on the table are more likely to have stunted (Yanti et al., 2023). When toddlers' daily menu composition is lacking in both quality and quantity, and when vegetable side dishes predominate in terms of frequency, they are considered to have limited access to food (Adelia et al., 2018). Lack of access to fulfilling food necessities is caused by low family income. Children from high-income families can buy various foods to meet their nutritional requirements. However, children from low-income families have monotonous/not-varied food consumption. Income level limits at family's purchasing power, which can lead to household food insecurity (Amalia & Mahmudiono, 2017). Food insecurity is very dangerous to toddlers' health (Schmeer & Piperata, 2017) and creates risky habits and mental health problems during the transition period to adolescence and adulthood (Heflin et al., 2019).

CONCLUSION

According to a meta-analysis of 14 publications, this systematic review showed that higher levels of household food security are associated with lower rates of stunting among Indonesian toddlers. Stunting is more likely to affect toddlers from homes with food poverty than those from households without food security. When everyone in a household has enough food to eat, the household is regarded as food secure. According to two studies, there was no correlation between household food security and the prevalence of stunting. As a result, citizens look to the government to enact policies and tactics that will boost the food security of American households and the health of children under the age of five. For these efforts to succeed, coordination between sectors is needed to accelerate and strengthen the implementation of these policies and strategies.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest in this research.

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DEVELOPMENT OF SEMIQUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE FOR IDENTIFICATION OF INDIGENOUS DIETARY PATTERNS IN PREDIABETIC ADULT WOMEN

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ABSTRACT

Dietary patterns influence the progression of prediabetes, so it is necessary to develop representative instruments for measuring eating habits, primarily indigenous dietary consumption. The research aimed to develop a semiquantitative food frequency questionnaire to identify indigenous dietary patterns in prediabetes sufferers in Gorontalo Regency. The research was a descriptive study that tested the validity and reliability of the food frequency questionnaire semiquantitative (FFQ-SQ). The research was conducted in Limboto Barat Health Center, based on the prevalence of diabetes mellitus. Selection samples using purposive sampling with 30 adult women. The validity test used internal validity Pearson correlation, while the reliability test used internal consistency Cronbach α . Comparison of average FFQ-SQ and recall 2 x 24-hours using Wilcoxon test. The results showed that 12 food groups consisted of 207 food items and indigenous food products with validity tests 123 items valid, 67 items invalid, and 17 items never consumed by the subject study in the previous month; the reliability test showed food group category low 10, moderate 5 and high 3. Comparative analysis of the energy and nutrient intake between FFQ-SQ with recall 2 x 24 hours showed significant differences in intake of carbohydrates, fiber, vitamin E, vitamin B1, folic acid, vitamin C, potassium and zinc (p -value < 0.005), and comparison of energy and nutrient intake between FFQ-SQ1 and FFQ-SQ2 showed no significant difference (p -value > 0.005). The conclusion was FFQ-SQ with 123 items of foods and derived products is valid and reliable for measuring the eating habits of prediabetic adult women.

Keywords: dietary patterns, FFQ-QS, prediabetic adult, reliability, validity

INTRODUCTION

Diabetes mellitus type-2 (T2DM) is a metabolic disease with symptoms that arise in a person due to an increase in blood glucose concentration. T2DM is a non-communicable disease (NCD) that prevalence continues to increase, which increases the risk of morbidity and mortality due to various complications (Soelistijo, Suastika, et al. 2021). In 2018, the prevalence of diabetes mellitus (DM) based on a doctor's diagnosis was 1.5% and in Gorontalo province was 1.7% at all ages. At the age of ≥ 15 years the prevalence of diabetes was 2.0% and in Gorontalo province was 2.4%. The incidence of DM prevalence increased compared to 2013, which was 1.5% (Kemenkes RI, 2013; Kemenkes RI, 2018). Half of DM sufferers are undiagnosed, only two-thirds are diagnosed with pharmacological or non-pharmacological treatment, and only one-third have improved metabolic control (Perkeni 2019).

Since 2014 the American Diabetes Association (ADA) has recommended T2DM prevention spectrum by treating hyperglycemia conditions and establishing a diagnosis of prediabetes (ADA 2013; Soelistijo et al., 2021).

Eating habits are related to the progression of diabetes mellitus. Vegetable and fruit consumption negatively correlates with blood glucose (Nailufar, 2022). The recommended consumption of vegetables per day ≥ 400 g based on WHO/FAO recommendations on diet, nutritional care and prevention of chronic diseases (Nishida et al., 2004). Perkeni recommended fiber intake of 20–35 g per day, and for DM patients at least 14 g per 1000 kcal and whole grain consumption (Soelistijo et al., 2021).

Food sources of carbohydrates with various proportions of sugar, starch and fiber content affect the glycemic response. Frequent diet of sugary foods and drinks is positively correlated with

blood glucose (Nailufar, 2022). Dietary intake of sweet foods such as traditional cakes, biscuits, cakes, donuts and sweet snacks is significantly related to blood glucose level (Yunianto et al., 2019). Based on WHO recommendations, sugar intake is recommended at 5-10% of total energy intake (TEI), in adults and children under 5 years sugar consumption is not more than 50 g (total energy needs 2,000 kcal) and 27-40 g (WHO, 2015). While, based on recommendations from the Indonesian Ministry of Health, sugar intake is no more than 50 g per day (MOH, 2013). The description of consumption of risky foods such as SSB tends to be high in Gorontalo, namely consumption of ≥ 1 time per day sweet foods 40.9%, sweet drinks 56.1%, consumption of soft drinks, carbonated drinks 3.2%, consumption of energy drinks 3.5% (Kemenkes RI, 2018). The frequency of dietary sweetened condensed milk category is often 25.0% (Lasimpala et al., 2021).

The depiction of vegetable and fruit consumption in Gorontalo people is still low accompanied by high consumption of risky foods such as sugar sweet beverages (SSB). The proportion of fruit and vegetable consumption per day in a week < 5 servings per day 93.8% (MOH, 2018). The average fiber intake of adults is 15.3 ± 8.9 g per day far from the recommended fiber intake recommendation of 30 g per day (Permenkes RI, 2019; Nuryani et al., 2021).

Dietary diversity is crucial to maintaining health and preventing T2DM in the prediabetes group. The development of instruments that facilitate the measurement of dietary patterns of prediabetic adults is important, especially with the abundance of indigenous foods with diverse nutritional compositions.

In measuring dietary patterns, standardized measuring instruments are needed, this is to ensure the consistency of measurements and the accuracy of the data collected. A standard measuring instrument must meet psychometric criteria, namely validity and reliability. Questionnaire (measuring instrument) in general is a tool used to measure natural phenomena and observed social phenomena. Questionnaires play an important role in determining the quality of a study, because the validity of the data obtained will be largely determined by the quality of the questionnaire

used. So this study aims to develop a semi-quantitative food frequency questionnaire as an effort to identify indigenous dietary patterns in prediabetes patients in Gorontalo Regency.

METHOD

The study was a descriptive research by testing the validity and reliability of the food frequency questionnaire (FFQ-SQ) questionnaire. Research measured was quantitative, where the interpretation of study results based on statistical processed results was using computer applications.

The location of the study was determined purposively based on the criteria for the prevalence of people with diabetes mellitus in the working area of the Limboto Barat Health Center, Gorontalo Regency. The study was conducted in June – August 2023.

The subjects of the study were adult women with a total of 30 people (Siegel, 1992). The determination of the research samples were based on the minimum requirements for parametric statistic tests. Purposive sampling of subjects who meet the inclusion criteria. The inclusion criterias of the study was adult women, living in Gorontalo district, willing to participate in research and signing informed consent. The adult age group criteria are the adult age group of women ≥ 30 –60 years based on the criteria of the highest incidence of diabetes (Kemenkes, 2018). While the exclusion criteria of subjects was adult women with comorbidities/chronic diseases based on doctors diagnosis, pregnant and lactating women.

The measurement of research data included data on basic characteristics, eating habits. Dietary patterns were measured using 2 x 24 hours recall and FFQ-SQ. The dietary patterns questionnaire was using FFQ-SQ that was developed by making a list of food ingredients, based on a list of food ingredients composition and based on food availability that can be found in Gorontalo (Kemenkes, 2013). Food grouping ingredients were based on FFQ-SQ food grouping in prediabetes, diabetes mellitus and food grouping patients for adult community groups in Indonesia (Sarmiento, Riboldi, et al., 2013 ; Schwingshackl et al., 2017 ; Siddiqui, Zainal, Harun, & Ghadzi, 2019 ; Syauqy et al., 2021 ; Bazzano, Li, Joshipura, & Hu, 2008;

Basiak-Rasala, Rozanska, & Zatonska, 2019). In the preparation of food grouping, it would be compiled and developed in accordance with indigenous food and typical food products found in Gorontalo. Development of FFQ-SQ questionnaire in the form of 207 lists food items and processed indigenous food products found in Gorontalo. The food grouping list includes 18 food groupings consisting of 1) staple foods of cerealias and root vegetables - 10 items, 2) staple foods of flour products - 7 items, 3) animal protein of meat and poultry - 12 items, 4) animal protein of fish and seafood - 20 items, 5) vegetable protein of nuts and processed - 11 items, 6) milk vegetable protein and its processed products - 7 items, 7) factory processed food protein - 6 items, 8) light-colored vegetables - 14 items, 9) dark-colored vegetables 14 items, 10) fruits - 26 items, 11) sources of fats and oils as many as five items, 12) sugar, syrup and confectionery - 11 items, 13) seasonings of 12 items, 14) processed foods with oil 12 items of processed food products, 15) processed foods sources of protein nine items of processed foods, 16) processed foods sources of carbohydrates and nuts - 11 items, 17) snacks, as many as 12 items, 18) food and beverages packaged bottles or boxes as many as 8 items. Identification of indigenous food in this study base on 207 lists food consumption of the subject that can decrease or increase risk of diabetes such as staple food corn rice, cassava, and corn contain high fibers that can prevent diabetes and risky food such as food processing with high fat using coconut milk, sweet sugar food and beverages consumption that can increase risk of diabetes. Filling the FFQ-SQ instrument was carried out twice with a span of 1 week to compare the average intake of energy and nutrients. FFQ-SQ was also compared with the measurement results of the 2 x 24 hours recall questionnaire. FFQ-SQ was developed with the frequent dietary from grocery items every meal time (score 2.5), every day (score 1), 3 – 6 x per week (score 0.42), 1 – 2 x per week (score 0.14), rarely (score 0.04) and never (score 0). Filling out the FFQ-SQ questionnaire by asking for a list of food items and food consumed by research subjects within the last 1 month. The eating frequency score was grouped into frequent consumption if the score ≥ 0.43 and infrequent consumption if the

< score is 0.43 (Marks, Hughes, & van der Pols, 2006). Some indigenous foods and typical food products found in Gorontalo that are not found in the data processing application or in the list of food ingredients composition will be made standard recipes in accordance with recipes and processing standards in the local area to subsequently include the composition of energy and nutrients in the database of food intake data processors.

After the preparation of the list of food and beverage ingredients in the FFQ-SQ questionnaire, the validity and reliability of the FFQ-SQ questionnaire was tested. The internal validity test was a correlation between the score of food items and the total score using the Pearson correlation test, the correlation coefficient was using r count compared to r table (r table, $dp = n - 2 = 28$, r table = 0.333), while reliability test using internal measurement of cronbach consistency α with > category 0.9 high reliability, 0.7 – 0.9 high reliability, 0.5 – 0.7 moderate reliability, < 0.5 low reliability (Lee et al. 2002; Juliandi, 2008; Siddiqui et al. 2019). In the FFQ-SQ instrument, take measurements 2 times with range time of 1 week (Lee et al. 2002; Siddiqui et al. 2019). A Wilcoxon test was used to analyze the comparison between the average intake of energy and nutrients using a 2 x 24-hours recall questionnaire and FFQ-SQ and compare the average intake of FFQ-SQ1 with FFQ-SQ2 with p value = 0.005. Research subjects on the validity and reliability test were asked for qualitative advice and opinions regarding the list of food ingredients contained in the questionnaire, as well as recommendations for certain types of food commonly consumed but not included in the FFQ-SQ questionnaire list.

Data collection was carried out by filling out questionnaires, interviews and direct measurements. The time needed by research subjects in filling FFQ-SQ $\pm 30 - 45$ minutes. Processing and analysis of food intake data using Nutrisurvey, Microsoft Excel while validity and reliability analysis using static product and service solution (SPSS 16). This research has passed the review of the health ethical research commission of the Faculty of Nursing and Health, University of Muhammadiyah Semarang number 094/KE/07/2023.

RESULTS AND DISCUSSION

Table 1. Research subject characteristic

Characteristic	n	%
Age		
30 – 34	4	13.3
35 – 39	7	23.3
40 – 44	14	46.7
45 – 49	5	16.7
Marital status		
Married	29	96.7
Divorced	1	3.3
Education		
< 9 years	20	66.6
≥ 9 years	10	33.4
Partner’s education		
< 9 years	20	66.6
≥ 9 years	10	3.4
Occupation		
Civil servant	1	3.3
Private servant	2	6.7
Entrepreneur	3	10.0
Laborer	2	6.7
Housewife	22	73.3
Partner’s occupation		
Civil servant	1	3.3
Private servant	3	10.0
Entrepreneur	10	33.3
Laborer	16	53.4
Income		
< Minimum wage	25	83.3
≥ Minimum wage	5	16.7
Gestational Diabetes		
Yes	2	6.7
No	28	93.3
Historical hypertension		
Yes	9	30.0
No	21	70.0
Historical NCD		
Yes	4	13.3
No	26	86.7
Family historical diabetes		
Yes	1	3.3
No	29	96.7
Family historical NCD		
Yes	5	16.7
No	25	83.3

Note: NCD: non-communicable disease.

An overview of the characteristics of research respondents in the validity and reliability test of the FFQ-SQ questionnaire is shown in

Table 1. Based on age characteristics, it shows that the largest age group is in the 40 - 44 year age category, particularly 14 women (46.7%). Marital status with married status as many as 29 women (96.7%), ethnic group 30 women (100%) Gorontalo tribe, highest level of education at middle school and high school level, with 8 people each (26.7%), most of the respondents’ occupation are housewives (housewives), with amount of 22 women (73.3%) while the education of the partner, or the respondent’s husband, is mostly at elementary school level, particularly 13 people (43.3%) while the most of the respondent’s partner’s occupation is as a laborer, particularly 16 respondents (53.4%), with an income level below the regional minimum wage for Gorontalo province, 25 respondents (83.3%).

Ethnic characteristics and disease histories are shown in Table 1. The ethnic characteristics of the research subjects are Gorontalo ethnic, which has a very low smoking habit of only 3.3% and the subjects have no history of consuming alcoholic beverages. Results in the questionnaire reliability test study show that 2 people (6.7%) had a history of gestational diabetes, 9 people (30.0%) had a history of hypertension. Based on the history of non-communicable diseases, it shows that 4 people (13.3%) had a history of non-communicable diseases, meanwhile, 1 person (3.3%) had a history of non-communicable diseases experienced by family members with a history of diabetes mellitus. As for the type of history of non-communicable diseases experienced by research subjects, there were 5 respondents (16.7%) who had family members with a history of hypertension and gout. A history of non-communicable diseases in family members is also a risk factor for prediabetes in accordance with the Indonesian Prediabetes Risk Score (INA-PRISC) prediabetes risk factor enforcement criteria (Fujiati, Damanik, Bachtiar, Nurdin, & Ward, 2017).

Validity is the ability of a measuring instrument to measure what it should measure, and provide the true value of the variable being measured. The method used to measure the validity of the questionnaire is the Pearson correlation between the score of each item and the total score, better known as the internal validity test. Reliability is the ability of a measuring instrument

or questionnaire to provide consistent results, which can be done by analyzing Cronbach’s alpha values (Sinaga, 2017).

An overview of the list of food ingredients in the FFQ-SQ food grouping, validity, and reliability analysis is shown in Table 2. Grouping of FFQ-SQ food ingredients for patients with prediabetes, diabetes mellitus and food grouping for adult groups in Indonesia (Sarmiento et al., 2013 ; Schwingshackl et al., 2017 ; Siddiqui et al., 2019 ; Syauby et al., 2021). In preparing the next food grouping, it was arranged and developed according to indigenous food and typical food products found in Gorontalo. The staple food of cerealias and root vegetables shows that there are 2 food items

with a calculated *Pearson* r correlation value < r table, namely white rice (r = -0.087) and taro (r = -0.020). This is due to the research subjects’ habit of consuming white rice every day with an FFQ score of 1.547 (frequent consumption), resulting in data homogeneity, while the taro food item has an FFQ score of 0.014 (rare consumption). Meanwhile, the result of the reliability test with a Cronbach’s alpha value of 0.485 indicates low reliability. The staple food product flour shows 2 food items with calculated r values < r table, specifically noodles (r = -0.002) and pasta (r = 0.127) because these food items are very rarely consumed by respondents. The result of the reliability test analysis with a Cronbach’s alpha shows value of -0.285 which is low reliability.

Table 2. Analysis of validity and reliability test of FFQ-SQ

Food grouping	Food items	r	Signifincae (two tailed)	Cronbach's alpha	Validity	Reliability
Cerealia staple foods and root veg- etables	Corn rice, corn, cassava, potatoes, purple sweet po- tato, white sweet potato	0.5 – 0.7	< 0.05	0.485	Valid	Low
	Brown rice, yellow sweet potato	< 0.5	< 0.05	0.485	Valid	Low
	White rice, taro	< 0.5	> 0.05	0.485	Invalid	Low
Staple food flour products	Laksa/vermicelli, cereal, spaghetti	0.5 – 0.9	< 0.05	-0.285	Valid	Low
	Bread, sago	< 0.5	< 0.05	-0.285	Valid	Low
	Noodle, pasta	< 0.5	> 0.05	-0.285	Invalid	Low
Animal protein (meat and poultry)	Beef, goat meat, beef jerky, offal (liver, intestines, lungs, marrow), free-range chicken meat, duck, liver, chicken eggs,	0.5 – 0.9	< 0.05	0.424	Valid	Low
	Duck eggs, quail eggs	< 0.5	< 0.05	0.424	Valid	Low
	Broiler, gizzard	< 0.5	> 0.05	0.424	Invalid	Low
Animal protein (Fish and sea- food)	Shellfishes	0.5 – 0.9	< 0.05	0.326	Valid	Low
	Mackerel / oci fish, skipjack tuna, trevally, crab, shrimp, nike (largesnout goby) fish	< 0.5	< 0.05	0.326	Valid	Low
	Flying fish, snapper, rab- bitfish, tuna, mackerel, tilapia, milkfish, snakehead fish, catfish, wet anchovies, dried fish, dried anchovies, squid	< 0.5	> 0.05	0.326	Invalid	Low

Food grouping	Food items	r	Signifincae (two tailed)	Cronbach's alpha	Validity	Reliability
Vegetable protein (le-gumes and their derived products)	Tempeh, tofu, green beans	0.5 – 0.9	< 0.05	0.312	Valid	Low
	Peanuts	< 0.5	< 0.05	0.312	Valid	Low
	Cashew nuts, jack beans, almonds, red beans, green bean sprouts	< 0.5	> 0.05	0.312	Invalid	Low
	Melinjo, sunflower seed	-	-	-	-	-
Vegetable protein (milk and its derived products)	Fresh skimmed milk, fresh milk, cheese, ice cream	0.5 – 1.0	< 0.05	0.823	Valid	High
	Powdered milk, yoghurt	< 0.5	< 0.05	0.823	Valid	High
	Skimmed, powdered milk	-	-	-	-	-
Processed protein foods (manufactured products)	Canned fish, sausages, salted eggs	0.5 – 0.9	< 0.05	0.223	Valid	
	Canned corned beef, smoked beef, frozen sea-food	-	-	-	-	
Light colored vegetables	Cabbage, mustard greens, chayote, zucchini, yellow pumpkin, papaya flowers	0.5 – 0.9	< 0.05	0.606	Valid	
	Vegetables cucumber, carrot, bamboo sprouts, young jackfruit	< 0.5	< 0.05	0.606	Valid	
	Purple eggplant, young papaya, tomatoes	< 0.5	> 0.05	0.606	Invalid	
	Mushrooms	-	-	-	-	
Dark colored vegetables	Green spinach, red spinach, aibika leaves, long bean leaves, cassava leaves, broccoli	0.5 – 0.9	< 0.05	0.414	Valid	
	Kale, long beans	< 0.5	< 0.05	0.414	Valid	
	Melinjo leaves, lettuce, bok choy, fern vegetables	< 0.5	> 0.05	0.414	Invalid	
	Chinese okra, bitter melon	-	-	-	-	
Fruits	Apples, oranges, young coconuts, mangoes, papaya	0.5 – 0.9	< 0.05	0.593	Valid	
	Avocado, duku, durian, melon, pineapple, water-melon	< 0.5	< 0.05	0.593	Valid	
	Guava, water apple, ambarella, langsats, caven-dish/ambon banana, saba/kepok banana, uli banana, plantain, horn banana, rambutan, snake fruit, sapodilla	< 0.5	> 0.05	0.593	Invalid	
	Srikaya, soursop, breadfruit	-	-	-	-	
Fat and Oil Sources	Palm oil, coconut milk	0.5 – 0.9	< 0.05	-0.609	Valid	
	Olive oil	< 0.5	< 0.05	-0.609	Valid	
	Coconut oil, Margarine/ butter	< 0.5	> 0.05	-0.609	Invalid	

Food grouping	Food items	r	Signifincae (two tailed)	Cronbach's alpha	Validity	Reliability
Sugar, Syrup, and Confec- tionery	Rock sugar	0.5 – 0.9	< 0.05	-0.068	Valid	
	Sweetened condensed milk, chocolate, palm sugar, granulated sugar, palm sugar, syrup, liquid drinks, powdered drinks, jam, candy, tea, coffee	< 0.5	> 0.05	-0.068	Invalid	
Spices	Leeks, shallots, sauce	0.5 – 0.9	< 0.05	0.446	Valid	Low
	Cayenne pepper, shrimp paste, sweet soy sauce	< 0.5	< 0.05	0.446	Valid	Low
	Red chili peppers, green chili peppers, garlic, basil leaves, soy sauce	< 0.5	> 0.05	0.446	Invalid	Low
	Onion	-	-	-	-	-
Snack	Banana chips, peanut brittle, crackers, wafers, wafer rolls	0.5 – 0.9	< 0.05	0.570	Valid	Moderate
	Cassava chips, cookies, pia, sponge cake	< 0.5	< 0.05	0.570	Valid	Moderate
	Biscuit	< 0.5	> 0.05	0.570	Invalid	Moderate
	Emping, tempe chips	-	-	-	-	-
Packaged food and drink, bottles/carton	Coffee products, Dairy products, Tea, Pudding	0.5 – 0.9	< 0.05	0.585	Valid	Moderate
	Juice/fruit juice, ionic drinks	< 0.5	> 0.05	0.585	Invalid	Moderate
	Soy milk products, Soft drinks (soda)	-	-	-	-	-
Processed foods in oil	Crispy fried chicken, yel- low rice, fried stuffed tofu, fried tempeh, vegetable bakwan, fried banana	0.5 – 0.9	< 0.05	0.736	Valid	High
	Fried rice, fried noodles, corn fritter, Sabongi, Martabak egg, sweet Martabak	< 0.5	< 0.05	0.736	Valid	High
Processed food sources of protein	Meatballs, Coto, Chicken Soto, Woku fish, Iloni chicken	0.5 – 0.9	< 0.05	0.571	Valid	Moderate
	Chicken feet	< 0.5	< 0.05	0.571	Valid	Moderate
	Rib soup, sour sauce, tuna satay	< 0.5	> 0.05	0.571	Invalid	High
Processed foods sources of carbohy- drates and vegetables	Tinutuan, Gado – gado, Chicken noodles, Binte Biluhuta, Ilabulo, Lalampa,	0.5 – 0.9	< 0.05	0.767	Valid	High
	Bamboo rice, potungo veg- etables, dodol pocong, iced green banana	< 0.5	< 0.05	0.767	Valid	High
	Iced Brenebone	< 0.5	> 0.05	0.767	Invalid	High

There is a tendency for low reliability in the carbohydrate source food group due to the large list of food items, namely 17 food items with a varied distribution and generally infrequent consumption, except for consumption of white rice which is in the frequent category.

In the group of animal protein sources, meat, and poultry, there are 2 food items with calculated r values $< r$ table, namely broiler chicken ($r = 0.136$) and gizzards ($r = 0.219$), which could be because consumption of these foods is relatively rare. Reliability test shows Cronbach's alpha value of 0.424 which is a low reliability. In the group of animal protein sources, fish and seafood, there are 11 food items with a calculated r value $< r$ table, namely flying fish ($r = 0.215$), snapper ($r = 0.230$), rabbitfish ($r = 0.230$), tuna ($r = 0.264$), mackerel ($r = 0.174$), tilapia fish ($r = 0.283$), milkfish ($r = 0.221$), catfish ($r = -0.004$), wet anchovies ($r = 0.160$), dried anchovies ($r = 0.303$) and squid ($r = 0.236$), this could be due to the relatively rare consumption of these foodstuffs. Reliability test shows a Cronbach's alpha value of 0.326 which is also a low reliability. In the group of vegetable protein sources, there are nuts and their derived products shows 4 food items with a value of r count $< r$ table, namely *karo* beans ($r = 0.022$), almonds ($r = 0.194$), red beans ($r = 0.124$) and green bean sprouts ($r = 0.300$) because these food items are very rarely consumed by respondents. There were 2 food items that research subjects had never consumed in the last month, melinjo and sunflower seed. Reliability test shows Cronbach's alpha value of 0.312 which is a low reliability. Vegetable protein sources in the milk group and its derived products show that most of the food items have a calculated $r > r$ table, however, 1 food item has never been consumed by research subjects in the last 1 month, specifically powdered skim milk. Reliability test shows Cronbach's alpha value of 0.823 which is a high reliability. Manufactured processed protein foods showed 3 food product items that research subjects had never consumed in the last month, they are canned corned beef, smoked beef and frozen seafood. Reliability test shows Cronbach's alpha value of 0.223 which is a low reliability. Reliability test analysis shows a low reliability category, this could be due to the large list of food items, which are 56 food items

and processed products with a diverse distribution and generally infrequent consumption.

The habit of consuming vegetables and fruit has a good impact on the mornings of prediabetics. The list of vegetable group food ingredients was based on the types of vegetables that can be found in Gorontalo. Consumption of light-coloured vegetables shows 2 food items with calculated r values $< r$ table, particularly young papaya ($r = 0.215$) due to low consumption, tomatoes ($r = 0.251$), which could be caused by frequent consumption of the category resulting in data homogeneity for food items (FFQ-SQ score = 0.934), and indigenous food that is generally found in Gorontalo is with a spicy taste of cayenne pepper and tomato sauce (Nuryani, Muhdar IN, Ramadhani F, Paramata Y, Adi DI, 2021). There is 1 food item that has never been consumed by research subjects in the last one month, namely consumption of mushrooms. The reliability test shows Cronbach's alpha = 0.606, which is a moderate reliability. Validity is classified as moderate because almost all of the food items in the questionnaire have been consumed by research subjects in the last month. Hence, the heterogeneity of the data varies. Consumption of dark-coloured vegetables shows 4 food items with calculated r values $< r$ table, namely melinjo leaves ($r = 0.292$), lettuce ($r = 0.292$), pokchoy ($r = 0.082$) and fern vegetables ($r = 0.082$), this can be caused by the rarity of consumption category, resulting in homogeneity of data for food items (FFQ-SQ score = 0.002). Two food items are never consumed, namely gambas and pare or bitter melon. The reliability test shows a Cronbach's alpha value = 0.414, which is a low reliability. This could be due to the extensive list of food ingredients (14 items) and many items that are rarely consumed or never consumed. Analysis of fruit consumption shows 11 food items with calculated r values $< r$ table, namely guava ($r = 0.293$), ambarella/Spondias *dulcis* ($r = 0.234$), langsung ($r = 0.265$), cavendish banana ($r = 0.254$), saba banana ($r = 0.031$), uli banana ($r = 0.036$), plantain ($r = -0.064$), horn banana ($r = 0.182$), rambutan ($r = 0.035$), snake fruit ($r = 0.281$), and sapodilla ($r = 0.215$) could be due to the rarity of the consumption category. Meanwhile, there are three food items that the research subjects never consumed: sugar apple,

soursop and breadfruit. The reliability test shows Cronbach’s alpha = 0.593, which is a moderate reliability. This could be due to the large number of food items, resulting in the consumption of various fruits, even in small quantities.

The food group components that are very important in identifying risk factors for prediabetes are the consumption of risky foods such as sugar, salt, fat and local processed food products with high sugar and fat content and consumption by research subjects. In comparison, the risky foods group, such as consumption of processed foods, soft drinks, fats and sugars, has a lower effect on health status (Bhisma, Triastuti, Pikir, & Trissatharra, 2023). Consumption of fat and oil sources shows one food item with a calculated r value < r table, namely coconut oil (r = 0.171). The reliability test shows Cronbach’s alpha value = -0.609, indicating low reliability.

Food in syrup and confectionery shows only one food item with a calculated r value < r table, namely rock sugar (r = 0.939). Meanwhile, the reliability test has a Cronbach’s alpha value = -0.609, which shows low reliability. This could be due to the extensive list of food ingredients (11 items) and many food items that are consumed infrequently. Food types of buffer spices show

five items with a calculated r value < r table, namely red chili peppers (r = -0.012), green chili peppers (r = 0.016), garlic (r = 0.276), basil leaves (r = 0.173) and soy sauce. (r = 0.128). Onions are the one of spice item that has never been consumed in the last month. The reliability test has a Cronbach’s alpha value = 0.446, which indicates low reliability. Kitchen spices show five items with a calculated r value < r table, namely red chili peppers (r = -0.012), green chili peppers (r = 0.016), garlic (r = 0.276), basil leaves (r = 0.173) and soy sauce. (r = 0.128). There is 1 spice item that has never been consumed in the last month, namely onions. Meanwhile, the reliability test has a Cronbach’s alpha value = 0.446, which indicates low reliability. This could be due to the large number of food items with infrequent consumption by research subjects. In the snack type food group, almost all food items show a calculated r value > r table, but only chips and tempeh chips that have never been consumed in the last month. Meanwhile, the reliability test has a Cronbach’s alpha value = 0.570, which shows high reliability. This could be due to food product items for snacks, although there are many food items, the frequency of consumption is quite high and consumed by many research subjects. Analysis

Table 3. Comparative Analysis between intakes of FFQ-SQ and 2 x 24-hours Recall

Nutrients	2 x 24 h Recall	FFQ-SQ1	FFQ2	p-value*	p-value**
	Mean ± SD	Mean ± SD	Mean ± SD		
Energy (kcal)	1207.5 ± 493.6	1296.6 ± 514.0	1485.9 ± 620.3	0.054	0.127
Protein (g)	42.9 ± 13.7	45.0 ± 16.3	50.7 ± 23.3	0.586	0.249
Fat (g)	38.3 ± 20.2	38.6 ± 17.7	61.9 ± 94.4	0.967	0.399
Carbohydrate (g)	174.0 ± 108.8	196.1 ± 91.2	222.3 ± 80.7	0.012	0.069
Serat (g)	4.8 ± 4.3	11.0 ± 4.2	12.1 ± 6.6	<0.001	0.593
Cholesterol (mg)	134.5 ± 141.0	124.7 ± 78.3	125.8 ± 80.8	0.271	0.943
Vitamin A (µg)	965.3 ± 1585.0	111.9 ± 813.6	1331.9 ± 586.9	0.141	0.106
Carotene (µg)	0.213 ± 0.466	0.326 ± 0.488	0.323 ± 1.891	0.073	0.388
Vitamin E (mg)	2.496 ± 1.684	3.946 ± 1.858	3.953 ± 1.891	0.003	0.773
Vitamin B1 (mg)	0.406 ± 0.214	0.586 ± 0.311	0.603 ± 0.310	0.006	0.630
Folic acid (mg)	70.36 ± 71.81	163.7 ± 100.8	159.9 ± 94.2	<0.001	0.992
Vitamin C (mg)	30.63 ± 86.19	59.70 ± 47.62	50.04 ± 37.36	<0.001	0.339
Sodium (mg)	507.14 ± 697.02	649.55 ± 775.78	607.22 ± 498.26	0.360	0.845
Potassium (mg)	1363.0 ± 1275.6	2042.7 ± 937.4	2555.1 ± 1715.4	<0.001	0.245
Zn (mg)	3.65 ± 1.57	4.73 ± 1.93	5.20 ± 2.60	0.002	0.537

*) Wilcoxon test variable of 2 x 24 hours Recall
**) Wilcoxon test variable of FFQ-SQ1 and FFQ-SQ2

of food and beverage products packaged in bottles or cartons shows 2 products with calculated r values $< r$ table, particularly juice/fruit juice ($r = 0.063$) and electrolyte drinks ($r = 0.63$). Soy milk and soft drinks were two of many products listed in packaged processed food that were never consumed by the research subjects. The reliability test has a Cronbach's alpha value = 0.585, which shows moderate reliability. This could be due to the large number of food items consumed by many research subjects.

Processed food products, both traditional and typical of Gorontalo and other food products, can influence people's consumption habits, which impact controlling blood glucose. Processed food is divided into three food groups: processed food with oil, processed food as a source of protein, and processed food as a source of carbohydrates, vitamins and minerals. Egg martabak ($r = 0.078$) and sweet martabak ($r = 0.124$) were the two processed food items that have r values $< r$ table. The reliability test with Cronbach's alpha value = 0.736 shows high reliability, possibly due to the research subjects' frequent consumption of processed food snacks with oil, resulting in high FFQ-SQ scores. Processed food sources of protein show all processed food items with calculated r values $< r$ table and Cronbach's alpha = 0.571, which shows moderate reliability, which may be due to the variety of processed foods consumed by research subjects. Processed food sources of carbohydrates and vegetables show one processed food item with a calculated r value $< r$ table, namely Brenebone ice ($r = 0.248$). Cronbach's alpha analysis = 0.767 shows high reliability, possibly due to the research subjects' high consumption of processed foods. The processed food product items are a list of typical Gorontalo processed foods popular and widely consumed by research subjects.

The validity test uses a correlation test. The results of this study are almost the same as the results of previous research, which found variability in validity tests, namely the correlation coefficient FFQ1 and FFQ2 ($r = 0.58 - 0.92$), a moderate – high relationship based on the validity test on nutritional components ($r = 0.40 - 0.68$) (Liu et al., 2022). Research testing the validity of the FFQ questionnaire in Japanese society

shows varying validity values ($r = -0.12 - 0.86$) (Nanri, Fujiwara, Miyake, Kashino, & Mizoue, 2022). Likewise, the results of research testing the validity of the FFQ questionnaire in the adult group, with Pearson's correlation ($r = 0.58 - 0.90$) (Marques-Vidal et al., 2011). Another study that tested the validity of the FFQ-SQ showed that an increase in food consumption scores using the FFQ-SQ measurement positively correlated with an increase in biochemical biomarkers in metabolic syndrome sufferers, such as fasting blood glucose with fruit consumption ($r = 0.221$) and dessert consumption ($r = 0.229$), blood pressure with snack consumption ($r = 0.272$) (Nirdnoy et al., 2023).

A comparison of the average intake of energy and nutrients between the 2 x 24-hours recall and the FFQ-SQ is shown in Table 3. Wilcoxon analysis compared the average intake between the 2 x 24-hours recall with the FFQ-SQ and the average intake between FFQ-SQ1 and FFQ-SQ2. Comparison of energy and nutrient intake between FFQ-SQ and 2 x 24-hours recall shows that the average intake of energy, protein, fat, cholesterol, vitamin A, carotene and sodium shows the same results (p -value > 0.05). It shows that the FFQ-SQ is representative in describing energy and nutrient intake using a 2 x 24-hours recall questionnaire. However, there are significant differences in the average intake of carbohydrates, fiber, vitamin E, vitamin B1, folic acid, vitamin C, potassium and zinc when using the FFQ-SQ instrument and 2 x 24-hours recall. It could be because the FFQ-SQ questionnaire had already been adjusted with the indigenous Gorontalo foods source of fiber, vitamins and minerals, which consisted of 54 items of vegetables and fruits so that the subjects could name the foods they ate within the last month. The results of this study are almost the same as previous research findings, which showed that the comparison of macronutrient intake tended to be higher when measured using FFQ compared to recall for protein nutrients; however, there was no difference in energy, carbohydrate and fat intake (Marques-Vidal et al., 2011).

A comparison of energy and nutrient intake between the FFQ-SQ1 and FFQ-SQ2 questionnaires was taken with two measurements with a timespan of one week. The study

results showed no differences in energy intake, macronutrients or micronutrients between FFQ-SQ1 and FFQ-SQ2 measurements (p -value > 0.05). FFQ-SQ is a valid measuring tool for assessing energy intake, nutrients, food ingredients and processed food products based on local food menus, which can potentially increase the risk of prediabetes. Using the FFQ-SQ instrument is essential to examine unhealthy and risky eating habits so that recommendations can be given to at-risk community groups to prevent prediabetes. The unique characteristics of processed foods with various ingredient compositions and the use of spices on local menus can be analyzed using the FFQ-SQ questionnaire.

CONCLUSION

There are 12 food groups consisting of 207 items of food ingredients and local food products, which are generally found in Gorontalo with validity test results of 123 valid items, 67 items are invalid, and 17 items of food ingredients and processed food products have never been consumed by the subject within one month. Lastly, reliability test analysis shows low reliability on ten food group items, moderate reliability on five food group items, and high reliability on three food group items and processed food products. Comparative analysis of energy and nutrient intake between FFQ-SQ with 2 x 24-hours recall, there were significant differences in the average intake of carbohydrates, fiber, vitamin E, vitamin B1, folic acid, vitamin C, potassium and zinc (p -value < 0.005); meanwhile, a comparison of energy and nutrient intake between FFQ-SQ1 and FFQ-SQ2 showed that there was no significant difference in energy and nutrient intake (p -value > 0.005). It is recommended to develop the research instruments by comparing the average intake of energy and nutrients with food weighing and the need to develop instruments for measuring eating habits to identify risky food consumption habits, especially local food groups in several regions in Indonesia.

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NUTRITION STATUS AND NEUROPSYCHIATRIC DISORDERS IN INDONESIAN CHILDHOOD LUPUS: EXPERIENCE AT A SINGLE TERTIARY REFERRAL CENTER

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ABSTRACT

NPSLE diagnosis is still challenging because of many SLE-related and non-SLE-related processes that can be presented in patient. The report of NPSLE in Indonesia is still limited. This study aim to describe the clinical features, nutrition status, and laboratory characteristics of Neuropsychiatric Systemic Lupus Erythematosus (NPSLE) and compared to non NPSLE case in Indonesian children. The study is a retrospective cohort study. Data were collected from the complete medical record of Juvenile Systemic lupus Erythematosus (JSLE) patients 2016 - 2020 at the Allergy Immunology Outpatient clinic at Dr. Soetomo General Academic Hospital. We include all patients with ages ranging from age 0-18 years old with a diagnosis of Systemic lupus Erythematosus (SLE). The diagnosis fo SLE based on American College of Rheumatology (ACR) criteria 1997 and Neuropsychiatric (NP) manifestations were classified using the standardized nomenclature and case definitions for the 19 NP manifestations linked to SLE developed in 1999 by the ACR ad hoc Committee. Disease activity SLE was defined according to the American Mexican-Systemic Lupus Erythematosus Disease Activity Index (Mex-SLEDAI) criteria. Statistical analysis conducted in this study was descriptive analysis, paired T-test (NPSLE vs. non-NPSLE as the dependent variable), Fischer exact test, and Pearson Chi-square test using SPSS ver. 21. A total of 90 patients with juvenile SLE were enrolled, but only 71 patients were eligible as participants with complete medical records obtained. Mex-SLEDAI score was significantly higher on NPSLE compared to non-NPSLE ($p=0.001$). There are no significant differences of body height, body weight, and body mass index between each group ($p>0.05$). The incidence of NPSLE was 29.57%, with clinical main symptoms were delirium (33.33%), seizures (33.33%), and psychosis (14.29%). 33.33% MRI/CT-scan findings noted brain abnormalities with the most prevalent were ischemic (14.29%), hypodense lesion, brain atrophy, multiple lymphadenopathy, and transverse myelitis (4.76%). The higher disease activity in NP SLE indicates the needs to close observation, the higher patient adherence to medication, and more comprehensive management to achieve therapeutic success.

Keywords: juvenile, mortality, neuropsychiatry, SLE, symptoms

INTRODUCTION

Juvenile Systemic lupus Erythematosus (JSLE) involves a broad of organ manifestations, including nervous system manifestation, which is one of the major causes of morbidity and mortality. The involvement of nervous system manifests in the form of neuropsychiatric, known as Neuropsychiatric Systemic Lupus Erythematosus (NPSLE), with an incidence of approximately 20-45% in children with SLE (Harel et al., 2006; Yu et al., 2006). The incidence of NPSLE is common in juvenile SLE or childhood-onset, with clinical symptoms such as a decline in work memory, verbal memory, and speed processing which has negative effects on academic achievement, school attendance, and affected the quality of life (Zúñiga, 2014; Patrícia et

al., 2019). The symptoms of NPSLE vary involving the central and peripheral nervous system, from diffuse Central Nervous System (CNS) disorders, such as acute confusion, psychosis, anxiety, and depressive disorders, to focal CNS syndromes, such as seizures, cerebrovascular disease, chorea, and myelopathy (Benseler and Silverman, 2007; Patrícia et al., 2019).

Nutrition status in SLE patients play a significant role in the occurrence of the symptoms. The body mass index determined quality of life as well. SLE patients with overweight and obese nutrition status reported to have higher possibility to experience fatigue and other symptoms (Zhu et al., 2010). Therefore it is important to have understanding between BMI and SLE in children.

Until now, NPSLE diagnosis is still challenging because of many SLE-related and non-SLE-related processes that can be presented in patient. Several studies have reported the prevalence and clinical symptoms of NPSLE under the American College of Rheumatology (ACR) criteria (Harel et al., 2006; Yu et al., 2006; Benseler and Silverman, 2007; Costagliola et al., 2018). The report of NPSLE in Indonesia is still limited. Therefore we aim to investigate the involvement of neuropsychiatric manifestation in childhood-onset SLE or juvenile SLE in tertiary referral hospitals in Surabaya in order to increase the knowledge of NPSLE cases specifically in Indonesia. We describe the clinical features, nutrition status, and laboratory characteristics of Neuropsychiatric Systemic Lupus Erythematosus (NPSLE) and compared to non NPSLE case in Indonesian children.

METHODS

The study was a retrospective cohort which taking data from complete medical records of jSLE patients from allergy-immunology outpatient clinics and wards during 2016-2020 at Dr. Soetomo General Academic Hospital. The Health Research Ethics Committee of Dr. Soetomo Surabaya had approved the study (Ethical Clearance Number 0077/LOE/301.4.2/VII/2020). Patient less than 18 years old with complete data and fulfilled 4 of the 11 SLE criteria from the 1997 ACR were included in this study. Other causes of neurologic symptoms such as electrolyte disturbances, metabolic disorders, and infections were also excluded. Patients with NP manifestation prior to JSLE diagnosis of more than 6 months were also excluded from the analysis. Patient who experienced neuropsychiatric symptom more than 6 months prior to jSLE diagnosis were excluded from NPSLE case. Similarly, patients with only one neuropsychiatric symptom such as headache, mood disorder, or anxiety were not included in NPSLE cases as these are symptoms which often found in population and the most likely causes is not the SLE.

The data collected were demographic data including gender, age, age at first diagnosis, family history of autoimmunity, clinical symptoms, anthropometric measurement results, and

laboratory-radiology results. While the criteria used for the diagnosis of SLE were the 1997 ACR criteria. Disease activity was measured using the Mex-SLEDAI, and NPSLE symptoms were classified based on the case definition by the ACR ad hoc committee (Guzman et al., 1992; Aviña-Zubieta et al., 2007). Examination of antinuclear antibodies (ANA) by immunofluorescence with the (4',6-Diamidine-2'-phenylindole dihydrochloride) DAPI kit method and anti-dsDNA by enzyme-linked immunosorbent assay eBioscience kit (USA) were recorded.

Patients were divided into two groups, namely the non-NPSLE and NPSLE groups. Both group compared using statistical analysis include Levene's test and normality test, descriptive analysis, paired T-test (NPSLE vs. non-NPSLE as the dependent variable), Fischer exact test, and Pearson Chi-square test using SPSS version 21.

RESULTS AND DISCUSSIONS

A total of 71 patients were collected in this study and 21 people (29.57%) with NPSLE symptoms were found. Eleven out of 21 patients (52%) were diagnosed with NPSLE since their first admission to the hospital. The average age was 156.48 ± 25.82 months old or 13.03 ± 2.15 years old, the ratio between boys and girls was 13/58. The participant characteristics were summarized in Table 1.

This study found no significant difference on age (156.48 ± 25.82 months old vs. 143.76 ± 43.87 months old, $p=0.220$), body weight (39.09 ± 9.81 kg vs. 34.14 ± 13.71 kg, $p=0.161$), body height (143.21 ± 8.87 cm vs. 139.07 ± 22.88 cm, $p=0.429$), body mass index (17.82 ± 7.45 kg/cm² vs. 16.98 ± 1.28 kg/cm², $p=0.748$), duration of the symptoms (186.65 ± 499.38 days vs. 71.50 ± 84.60 days, $p=0.140$), C3 (62.75 ± 36.09 units vs. 52.89 ± 37.67 units, $p=0.314$), ANA test (181.68 ± 161.69 units vs. 193.21 ± 140.68 units, $p=0.772$) and anti dsDNA (418.21 ± 350.58 units vs. 527.81 ± 562.55 units, $p=0.437$), hemoglobin (9.28 ± 3.35 mg/dL vs. 10.90 ± 11.43 mg/dL, $p=0.528$), white blood cells ($476.00 \pm 7,408.68/\text{mm}^3$ vs. $6,830.00 \pm 2,990.80/\text{mm}^3$, $p=0.184$) and platelet ($284,380.95 \pm 193,571.81/\text{mm}^3$ vs. $226,719.80 \pm 126,975.94/\text{mm}^3$, $p=0.142$). Only the Mex-SLEDAI score

showed a significant difference between NPSLE with non-NPSLE (11.28 ± 4.36 vs. 6.68 ± 3.53 , $p<0.001$).

There was no significant difference in the gender distribution of both groups ($p=0.319$). Boys to girls ratio was 2:19 on NPSLE and 11:29 on the non-NPSLE group. The most frequent symptoms of NPSLE were fever (42.86%) followed by rash (14.29%), pale and joint or bone pain (9.53%). Swollen, dizziness, and seizure only account for 4.76%. While on non-NPSLE participants, the most frequent symptom was rash (26%), fever 22%, pale (20%), joint or bone pain (10%), swollen (6%), bleeding, and rash (4%). Other symptoms, icterus, personality changes, fever, seizures, and rash and ulcer account for 2%. There is no significant difference in the main complaint incidence in both

groups ($p=0.252$). Also, we found no significant difference in the disease outcome ($p=0.397$).

The clinical symptoms and MRI/CT-scan findings of NPSLE were summarized in Table 2. The most prevalent clinical symptoms of NPSLE were delirium (33.33%), seizures (33.33%), and psychosis (14.29%). While Parkinson’s, dizziness, depression, and eye problems account for 9.52%. Vasculitis, paraplegia, epilepsy, and sleep disorders incidence was 4.76%.

Not all patients undergo CT scans and MRI/CT-scan. The abnormalities were detected in 7 (33.33%) subjects with NPSLE. The most frequent was ischaemic (14.29%), such as a hypodense lesion, brain atrophy, multiple lymphadenopathies, and transverse myelitis incidence was found in 1 patient (4.76%). Thirty-eight percent of NPSLE children had normal Magnetic Resonance Imaging (MRI) or Computed Tomography Scan (CT-scan) results.

Table 1. Subject Characteristics

Characteristic	NPSLE (n=21)	Non-NPSLE (n=50)	p
Age, month (mean \pm SD)	156.48 \pm 25.82	143.76 \pm 43.87	0.220
Gender, (n (%))			0.319
- Boys	2 (9.53)	11 (22)	
- Girls	19 (90.47)	39 (78)	
Body weight, kg (mean \pm SD)	39.09 \pm 9.81	34.14 \pm 13.71	0.161
Body Height, cm (mean \pm SD)	143.21 \pm 8.87	139.07 \pm 22.88	0.429
Body Mass Index, kg/cm ² (mean \pm SD)	17.82 \pm 7.45	16.98 \pm 1.28	0.748
Complaints, (n (%))			0.252
- Fever	9 (42.86)	11 (22.00)	
- Pale	2 (9.53)	10 (20.00)	
- Swollen	1 (4.76)	3 (6.00)	
- Dizziness	1 (4.76)	0 (0.00)	
- Bleeding	0 (0.00)	2 (4.00)	
- Rash	3 (14.29)	13 (26.00)	
- Seizure	1 (4.76)	0 (0.00)	
- Joint/bone pain	2 (9.53)	5 (10.00)	
- Breathless	0 (0.00)	2 (4.00)	
- Icterus	0 (0.00)	1 (2.00)	
- Personality changes	0 (0.00)	1 (2.00)	
- Fever, seizure	0 (0.00)	1 (2.00)	
- Pale, joint or bone pain	1 (4.76)	0 (0.00)	
- Rash, ulcer	0 (0.00)	1 (2.00)	
- Swollen, seizure	1 (4.76)	0 (0.00)	
Duration of the symptoms, days (mean \pm SD)	186.65 \pm 499.38	71.50 \pm 84.60	0.140
C3, unit (mean \pm SD)	62.75 \pm 36.09	52.89 \pm 37.67	0.314
ANA Test, unit (mean \pm SD)	181.68 \pm 161.69	193.21 \pm 140.68	0.772
Anti-dsDNA, unit (mean \pm SD)	418.21 \pm 350.58	527.81 \pm 562.55	0.437

Characteristic	NPSLE (n=21)	Non-NPSLE (n=50)	p
Hemoglobin (HB), mg/dL (mean ± SD)	9.28 ± 3.35	10.90 ± 11.43	0.528
White Blood Cells (WBC), /mm ³ (mean ± SD)	8,476.00 ± 7,408.68	6,830.00 ± 2,990.80	0.184
Platelet, /mm ³ (mean ± SD)	284,380.95 ± 193,571.81	226,719.80 ± 126,975.94	0.142
MEX SLEDAI Score	11.28 ± 4.36	6.68 ± 3.53	0.001
Anemia, (n (%))	15 (71.43)	43 (86.00)	0.184
Leucopenia, (n (%))	7 (33.33)	10 (20.00)	0.240
Thrombocytopenia, (n (%))	2 (9.52)	6 (12.00)	1.000
Outcomes, (n (%))			0.397
- Remission	11 (52.38)	23 (46.00)	
- Death	7 (33.33)	5 (10.00)	
- Lupus nephritis complication	2 (9.52)	10 (20.00)	
- Other complication	1 (4.76)	2 (4.00)	
- Loss of follow-up	0 (0.00)	10 (20.00)	

In this study, there are no significant differences in nutritional status between NPSLE and non-NPSLE. But if we see to the category of the BMI, most of the patients are included as underweight, with BMI less than 25 kg/m². SLE patients with active disease are mostly have rapid and severe weight loss which then lead to undernutrition. Study in Brazil showed 70.1% patients with autoimmune disease are undernutrition (Waitzberg, Caiaffa and Correia, 2001). In the case of SLE, there are still no well understanding of the factors which resulting to undernutrition, because it can be coming from genetic, environmental, and hormonal factors (Pocovi-Gerardino et al., 2018; Correa-Rodríguez et al., 2019). But, it is important to study about the quality of diet because it may impact to the undernutrition status in patients, which then resulting to a more severe condition namely anemia, low bone mineral density, cardiovascular diseases, and many more (Borges et al., 2012; Ahn et al., 2018).

Several studies have been conducted to investigate the incidence of NPSLE, with varied results, ranging from 2% to 95%, which was similar to this result (Yu et al., 2006; Benseler and Silverman, 2007; Muscal and Brey, 2010; Giani et al., 2021). Central Nervous System (CNS) is the most affected compared to the peripheral and autonomic nervous system (Harel et al., 2006; Benseler and Silverman, 2007) and more severe in children patients compared to adult patients, which is the major causes of mortality and morbidity, such as resulting in organ damage with poor prognosis (Jeltsch-david and Muller, 2014; Khajezadeh et

al., 2018). The impact counted for more than 90% of NPSLE cases (Kivity et al., 2015; Sassi et al., 2017). Many factors contribute to the inconsistency of the incidence of NPSLE, including the study design, study methodology, selection criteria, and the scarcity of several neuropsychiatric syndrome (Jeltsch-david and Muller, 2014).

The causes of NPSLE are still unclear, but it is suggested that antibody-mediated neurotoxicity, vasculopathy due to anti-phospholipid (aPL) antibodies and cytokine-induced neurotoxicity, and the loss of neuroplasticity are the highest possible causes, which affected directly to gray and white matter structures (Muscal et al., 2010; Kivity et al., 2015). The pathological mechanism of NPSLE started by a subset of cross-reactive anti-double-stranded deoxyribonucleic acid (DNA) autoantibodies binding to NR2 glutamate receptor, which then triggered neuronal death due to glutamate release and excitatory cell death (Muscal and Brey, 2010). Neuropsychiatric manifestation of SLE was associated with the disease activity, which is in line with these findings (Yoon et al., 2019). Mex-SLEDAI score was significantly higher in NPSLE. Others found that NPSLE participants suffered from organ damage at a higher rate (The Systemic Lupus International Collaborating Clinics (SLICC) / ACR Damage Index), compared to non-NPSLE (Jo et al., 2002). Anti-glutamate receptor antibodies may also play a role in cognitive dysfunction and psychiatric disease in patients with SLE. Degiorgio first demonstrated that a subset of lupus anti-DNA antibodies cross-react with the NR2 glutamate receptor in patients with SLE (Degiorgio et al., 2001).

Table 2. Clinical symptoms

Clinical symptoms	n (%)	MRI/CT-scan	n (%)
Parkinson	2 (9.52)	Hypodense lesion	1 (4.76)
Moody	2 (9.52)	Brain atrophy	1 (4.76)
Delirium	7 (33.33)	Multiple lymphadenopathies	1 (4.76)
Seizure	7 (33.33)	Ischaemic	3 (14.29)
Dizzy	2 (9.52)	Transverse myelitis	1 (4.76)
Vasculitis	1 (4.76)	Normal MRI/CT-Scan	8 (38.10)
Psychosis	3 (14.29)	Not enrolled	6 (28.57)
Paraplegia	1 (4.76)		
Epilepsy	1 (4.76)		
Sleep disorders	1 (4.76)		
Depression	2 (9.52)		
Eye problems	2 (9.52)		
No symptoms	3 (14.29)		

The onset of NPSLE in the previous study varies from 12 – 15.2 years old (Carolyn, Doughty and Athreya, 1981; Harel et al., 2006; Yu et al., 2006; Costagliola et al., 2018). A study conducted in Bangkok noted the onset of NPSLE was started from 13 years old which was similar to this study (Suwanpakdee and Hostpital, 2017). Several studies noted the onset of SLE around the age of 16 to 55 years old, or childbearing age. Many factors are involved in the etiology and pathogenesis of SLE, including hormonal change (Sassi et al., 2017). It was proposed that hormonal changes, including steroid sex, which might be constituted of an endogenous milieu that promotes the development of SLE in a susceptible individual, accompanied by environmental factors (Jeltsch-david and Muller, 2014).

The most frequent symptoms of the study were seizure and delirium, which is in line with other findings (Khajezadeh et al., 2018). The incidence of seizure was 7-30% of NPSLE participants as the mark of the earliest CNS manifestation and focal CNS syndromes (Jeltsch-david and Muller, 2014; Salman-monte, Monfort and Carri, 2021). But previous study stated the incidence of seizures was more than 40% (Harel et al., 2006; Yu et al., 2006). Generalized, seizures tend to be associated with disease activity, and it was important to exclude the causes of seizures (Kivity et al., 2015). Seizures are slightly more prevalent at the onset and during disease in childhood SLE compared to adult SLE (Salman-monte, Monfort and Carri, 2021).

Delirium or “acute confusional state”, known as Lupus cerebritis, has an incidence ranging from 0 to 7%, which is much lower than this study, which suggest the involvement of primary CNS with the disease (Turkel, Miller and Reiff, 2001; Kivity et al., 2015). A study in Malaysia counted the incidence of delirium as 24.2%, and 26% in China, which was lower than this study (Fan et al., 2014).

Psychosis was the primary manifestation of SLE and part of the criteria for “neurologic disorder”, and mostly present due to corticosteroids medication (immunosuppressive therapy) and active lupus, especially if the dose is high, so it is important to distinguish the cause of psychosis by reducing the dose of corticosteroids and monitoring lupus activity, which it affects 2-11% of the patients (Turkel, Miller and Reiff, 2001; Mak et al., 2008; Kivity et al., 2015). The other studies showed that the incidence of psychosis was 18.5-24.2% which was higher than this study (Saunders et al., 2016). It also indicates active CNS disease in approximately 60% of cases (Turkel, Miller and Reiff, 2001).

NPSLE is complicated and difficult to diagnose due to the variation of neurologic manifestations and confounding disorders, so that brain imaging is important to determine the diagnosis (Jr et al., 2010). Anatomical cerebral abnormalities were found in 25% of SLE patients (Sibbitt, Sibbitt and Brooks, 1999). The incidence of abnormal MRI varies between 19-75% in

NPSLE (Jeong et al., 2015). MRI/CT-scan with no abnormal findings in NPSLE was 59% which is much higher than this study (38.10%) (Saunders et al., 2016). However, it has been noted that the incidence of normal brain MRI in SLE ranged between 34 - 41% (Cannerfelt, Nystedt and Jo, 2018). An abnormal finding was found in 33% of adult NPSLE, which is similar to this study (Steinlin et al., 1995).

Ischemic was predominant in this study, it happened in 16.7% of adult NPSLE, which was similar to this study (Zaky et al., 2015). Ischemic might be mediated by the overproduction of pro-inflammatory cytokines which damaging the brain-blood barrier (BBB) in different sites, and further, representing various neuropsychiatric symptoms because auto-antibody entering the brain. Pro-inflammatory responses by monocytes or endothelial cells might be induced by Anti-ribosomal-P and anti-NR2 antibodies (Kivity et al., 2015). Due to the high incidence of ischemic, SLE is an independent risk factor for stroke, which the incidence was 15%. It was noted that patient aged more than 50 years old had a higher risk of stroke, 1.5-fold in the first 5 years of the disease (Sibbitt, Sibbitt and Brooks, 1999).

MRI examination showed brain lesion in NPSLE patients (Sibbitt, Sibbitt and Brooks, 1999). It was stated that the reduction of N-acetyl aspartic acid (NAA) in NPSLE correlates with small focal lesions elsewhere in the brain as seen on MRI. The reduction of NAA is also seen in patients with generalized seizures, psychosis, or delirium. Low levels of NAA due to cytotoxic effects (e.g. antineuronal antibodies, cytokines, or small molecule neurocytotoxins) (Jr et al., 2010). Hypodense lesion happened to 1 (4.76%) NPSLE participant.

The incidence of brain atrophy occurs in 8.7 to 32% of patients with SLE, the incidence of brain atrophy in this study was still in this range (Zaky et al., 2015). Other stated higher incidence of brain atrophy in NPSLE patients, ranging from 19–43% (Cannerfelt, Nystedt and Jo, 2018). It is the most common finding in NPSLE (Cannerfelt, Nystedt and Jo, 2018; Silvagni et al., 2022). The disruption of BBB might be the most common cause of dysfunction of the blood-cerebrospinal fluid barrier and causing brain atrophy due to the

loss of parenchymal structures (Silvagni et al., 2022). White matter lesions and brain atrophy have correlation to cognitive dysfunction, but the specific domain has not been specified (Cannerfelt, Nystedt and Jo, 2018). Atrophy developed slowly over several years, and involved the frontotemporal regions (gray and white matter). It is correlated with the disease activity and the duration of the disease, complement deficiency, aPL antibodies, and the duration of corticosteroid treatment (Sarbu and Sarbu, 2020).

Lymph nodes are the peripheral organs. Brain tissue reactive antibodies are synthesized in the CNS and peripheral organs, including lymph nodes, and crossing the BBB, which was affecting the neurons (Magro-checa, Steup-beekman and Huizinga, 2018). The prevalence of lymphadenopathy in SLE is still unknown, although several cases report that generalized lymphadenopathy is the manifestation of SLE (Magro-checa, Steup-beekman and Huizinga, 2018). This study found the incidence of lymphadenopathy in 1 subject. The incidence of myelopathy was rare in SLE with severe effects which estimated a prevalence of 1%. Acute transverse myelitis is caused by an inflammatory process (Monahan et al., 2020). Our study found 1 subject with transverse myelitis, with rapid onset of motor, sensory and autonomic dysfunction at a spinal cord level.

CONCLUSION

This study found no significant difference on age, body weight, body height, body mass index, duration of the symptoms, C3, ANA test and anti dsDNA, hemoglobin, white blood cells and platelet ($p>0.05$). Only the Mex-SLEDAI score showed a significant difference between NPSLE with non-NPSLE ($p<0.001$). NPSLE in Indonesian children is quite rare with varied clinical manifestations. The higher disease activity in NP SLE indicates the need for close observation, higher patient adherence to medication, and more comprehensive management to achieve therapeutic success.

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THE EFFECT OF PROVIDING EDUCATIONAL MEDIA ON ANEMIA AND NUTRITION ON THE PREVENTION OF ANEMIA IN YOUNG WOMEN IN BEKASI CITY

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ABSTRACT

Adolescents need to pay attention to the fulfillment of their nutritional needs at this time because with this transition period there is an increase in the need for nutrients to support physical and psychological growth and development. One of the nutritional problems faced by adolescents is anemia. The prevalence of young women suffering from anemia in Bekasi City in 2020 is 26.4%. The purpose of this study was to determine the effect of providing educational media on anemia and nutrition on the prevention of anemia in young women in Bekasi City. This study was a pre-experimental study with pretest and posttest designs. The sample in this study was 60 young women who were divided into three groups with 20 young women in each group, each group had an intervention using different media, namely leaflet media, animated video, and QuizWhizzer game innovation media. The results of the paired t-test in the three groups showed a change in the increase in knowledge in each education group, with p-value in the leaflet group of 0.000, in the video animation group of 0.017, and the quiz whizzer game group of 0.012. Based on the results of this study, it can be concluded that the provision of educational media about anemia and nutrition has an influence on the prevention of anemia in young women.

Keywords: education, media, knowledge, anemia, women

INTRODUCTION

Adolescence is the transition period of a person from child to adult. Adolescence is a very important period because at this time there are many changes both biologically, psychologically and physically. Physical changes are very rapid growth (Adolescence Growth Spurt), which requires more nutrients. In addition to supporting physical growth and development, more nutrients are also needed for children's psychological growth and development. Ignorance of the fulfillment of these nutritional needs will result in various nutritional problems, such as undernutrition, overnutrition, and micronutrient deficiencies.

One of the nutritional problems faced by adolescents is anemia. Anemia is a condition where the hemoglobin (Hb) level in the blood is below the normal threshold (12 g/dL). Riskesdas 2013 data shows that the prevalence of anemia in WUS aged 15 years and over is 22.7%, while pregnant women are 37.1% (Ministry of Health, 2018). The World Health Organization (WHO) shows data on the prevalence of anemia in women of childbearing age in 2019 in Indonesia, which

is 31.2%. Based on data from the Health Office (DHO) 2017, the proportion of adolescent anemia in Bekasi City was 26.4%. (Bekasi City Health Office, 2020). Young women have a greater risk of developing anemia than young men, because young women experience menstruation every month and are still in the growth stage so they need more iron intake. In addition, young women often diet to maintain or beautify their body shape so that they consume little food which results in their nutritional needs not being met. Young women are usually very concerned about body shape, so many limit food consumption such as on a vegetarian diet (Simanungkalit & Simarmata, 2019). In the short term, anemia will have a direct impact on young women, including 5L, often complaining of dizziness, foggy eyes, eyelids, lips, hands, and skin look pale. While in the long term anemia will have an impact on young women and other women of childbearing age (WUS) in the future because women will experience pregnancy and have children. Young women who experience anemia can be more severe during pregnancy because at that time they need more iron and if not treated it

will have a negative impact on the fetus and the mother (Apriyanti, 2019).

Although anemia is known to be caused by iron deficiency in the body, vitamin B12 and folic acid deficiency can also cause anemia. Vitamin B12 and folic acid function in the formation of cell nucleus DNA and myelin formation. If vitamin B12 and folic acid are deficient in the body, it will cause disruption of DNA synthesis in the erythroblast nucleus so that the maturation of the nucleus is slower and the chromatin is looser resulting in cells becoming larger due to slow cell division called megaloblast cells which will later cause megaloblastic anemia (Kurniasari et al., 2018).

One of the government's solutions in overcoming and preventing anemia is by providing Blood Addition Tablets (TTD) to young women and pregnant women by the Puskesmas Office, in the form of 4 tablets consumed for 1 month, each 1 tablet consumed for 1 week (Putri et al., 2017). The Indonesian Ministry of Health created a program called PPAGB (Prevention of Iron Nutrition Anemia) and has been implemented in Bekasi City since 2010 until now with previous updates to the procedure for taking blood supplement tablets in 2016 to prevent anemia in young women or schoolgirls in schools (Murnariswari, 2021). However, TTD consumption in young women is known to be low. According to the 2018 Riskesdas, the percentage of young women who consumed TTD >52 items was only 1.4%. This low TTD consumption can be due to the fact that not all schools have implemented the PPAGB program and there are still many female students who do not want to take TTD because they have low knowledge about anemia.

Providing education to young women can be another alternative in preventing anemia. Providing education must be interesting so that it can generate interest and interest in young women. According to Marfuah et al. (2016), education provided to young women affects the motivation of young women to consume iron, especially during menstruation to prevent anemia. The education provided can be in the form of knowledge about anemia and nutrition with the benefit that young women understand

about anemia and a healthy diet and lifestyle in everyday life.

Providing education can be done with various media as tools, such as leaflets, animated videos, and games. This is because providing education using these media is more attractive to young women because it is easier to learn and understand, and interesting. In addition, using these media makes it easier for young women to access the education provided through their cellphones without the need to carry a lot of items. The use of media in providing education can help the information provided to be conveyed better and easier to understand. Based on the description above, the purpose of this study is to determine the effect of providing educational media on anemia and nutrition on the prevention of anemia in young women in Bekasi City.

METHOD

This research is a pre experimental research with pretest and posttest design. This research was conducted by providing online-based education to young women in Bekasi City which was carried out in April 2022. The sample in this study amounted to 60 young women who were divided into three groups with each group totaling 20 young women. The sample was divided into three intervention groups and given three different educational media, namely 2-dimensional leaflet educational media, 3-dimensional animated video educational media, and QuizWhizzer game innovation media. The three media were used because they were easy to access, easy to learn and understand, and interesting.

The three media were made by researchers with the help of the Canva application, Youtube, and the QuizWhizzer website. Each media has the same discussion content and there is no difference. The discussion contained in the media is the definition of anemia, why young women are prone to anemia, causes of anemia, signs and symptoms of anemia, the impact of anemia, how to prevent and treat anemia, and substances that need to be limited in consumption because they can inhibit iron absorption.

The distribution of samples to each group was randomized until each group had 20 samples each. Samples were obtained using purposive sampling

technique, which is a technique of sampling data sources with certain considerations. The use of purposive sampling technique is because there are certain considerations or criteria that must be met by the samples used in this study. The inclusion criteria in this study were young women aged 12-22 years, domiciled in Bekasi city, and willing to participate in this study, while the exclusion criteria in this study were young women who did not fill out the pretest and/or posttest.

Data were collected using a questionnaire containing 25 questions about anemia, such as definition, impact, signs and symptoms, causes, prevention and treatment of anemia, and substances that need to be limited in consumption. The questionnaire used had previously been validated by the researcher. Overall, the intervention of each group was conducted once on the same day and within approximately 2 hours. Completion of the pretest was carried out 30 minutes before being given education about anemia and the posttest was carried out 30 minutes after being given education about anemia. The assessment carried out based on the questionnaire is that if the respondent answers correctly on one question, it will score 1 point, while if the respondent answers incorrectly, it will score 0 points. Data analysis used a statistical analysis program, namely SPSS version 26 with the Kolmogorov Smirnov test for normality test and Paired Sample T-Test test to see whether there was a change in knowledge level between before and after education.

RESULT AND DISCUSSION

Characteristics of Respondents

This study was conducted on 60 young women who were divided into three groups, each group consisting of 20 young women. The characteristics of all respondents who participated in this study consisting of age, body weight (BW), and height are shown in table 1.

Table 1 above shows that the characteristics of respondents vary from age, weight, height, and BMI. The leaflet group showed that the age characteristics of the youngest respondent were 13 years old and the oldest was 22 years old, with body weight characteristics having a mean of 53.35 ± 7.995 kg, having a mean height of

Table 1. Characteristics of Respondents

Characteristics	Average \pm SD	Min – Max
Leaflet Group		
Age	17.55 ± 3.103	13 – 22
Body Weight	53.35 ± 7.995	35 – 64
Height	157.45 ± 4.322	149 – 165
BMI	21.52 ± 3.047	13.01 – 26.14
BMI for Age	-0.36 ± 1.206	(-3.00) – (+1.00)
Animation Video Group		
Age	15.65 ± 2.033	14 – 20
Body Weight	46.40 ± 6.636	35 – 65
Height	157.10 ± 2.808	154 – 163
BMI	18.75 ± 2.531	15.00 – 27.00
BMI for Age	-0.59 ± 1.004	(-2.00) – (+2.00)
QuizWhizzer Group		
Age	18.05 ± 2.892	14 – 22
Body Weight	51.05 ± 12.791	38 – 82
Height	155.15 ± 5.029	140 – 161
BMI	20.90 ± 4.364	16.00 – 32.00
BMI for Age	-0.60 ± 0.516	(-1.00) – (0.00)

157.45 ± 4.322 cm, BMI for respondents over 18 years of age (>18 years) at a minimum of 13.01 and a maximum of 26.14, and BMI/A for respondents with an age range of 13 to 18 years at a minimum of -3 SD and a maximum of +1 SD. The animation video group had respondents with the characteristics of the youngest age of 14 years old and the oldest age of 20 years old, body weight characteristics had a mean of 46.40 ± 6.636 kg and height characteristics with a mean of 157.10 ± 2.808 cm. The BMI of respondents aged over 18 years had a minimum of 14.56 and a maximum of 27.05, and the BMI/A of respondents aged 14 years to 18 years had a minimum of -2 SD and a maximum of +2 SD. The QuizWhizzer group shows characteristics according to the age of respondents varying with a minimum age of 14 years old and a maximum age of 22 years old, having body weight characteristics with an average of 51.05 ± 12.791 kg, height characteristics with an average of 155.15 ± 5.029 cm, a minimum BMI of 16.00 and a maximum of 32.00, and a minimum BMI/A of -1 SD and a maximum of 0 SD. The nutritional status of ayoung women can be seen from the body mass index (BMI) and body mass index according to age (BMI/A).

The age of respondents in this study varied, with the youngest being 12 years old and the oldest being 22 years old. Taking the age range in young

women has previously been adjusted to the age category issued by the Indonesian Ministry of Health as explained in Amin (2017) research that the adolescent category is divided into two periods, namely early adolescence (12 - 16 years) and late adolescence (17 - 25 years). Therefore, to determine the nutritional status of respondents, they were checked based on BMI and BMI/A. Based on the Regulation of the Minister of Health of the Republic of Indonesia (PMK RI) in 2020 concerning Child Anthropometric Standards, BMI/A for ages 5-18 years has 4 categories, namely undernutrition (-3 SD), good nutrition (-2 SD to $+1$ SD), overnutrition ($+1$ SD to $+2$ SD), and obesity ($> +2$ SD), meanwhile based on Kemenkes (2019), BMI has categories, namely the thin category (weight <17.0 and light $17.0 - 18.4$), normal ($18.5 - 25.0$), and obese (light $25.1 - 27.0$ and heavy >27). Adolescents with thin and fat categories have a higher risk of anemia. Young women must maintain their weight so that their nutritional status remains good. Previous studies state that there is a positive relationship between nutritional status and the incidence of anemia. Underweight nutritional status describes chronic energy deficiency (CED) in the individual. In addition, overweight and obese nutritional status also have a relationship with the incidence of anemia, where there is increased inflammation in the adipose tissue of obese individuals so that hepcidin production increases and causes iron absorption to be inhibited (Lutfitasari, 2021). However, there are other studies which state that there is no relationship between BMI and the incidence of anemia in young women (Estri & Cahyaningtyas, 2021). The absence of a relationship between BMI and the incidence of anemia is because in addition to Hb levels, BMI is also influenced by many other factors, such as infection, lifestyle changes, liver enlargement, low nutrient intake so that growth is inhibited and causes weight loss and is not ideal.

The Effect of Leaflet Education on Anemia and Nutrition on the Knowledge of Young Women

The paired T-test analysis was used to determine changes in knowledge improvement before and after education using leaflets with a sample of 20 young women. If the analysis results show a p-value > 0.05 , it means that there is no



Figure 1. Leaflet on Anemia and Nutrition

change in improvement. Conversely, if the results of the analysis show a p-value <0.05 then there is a change in improvement in the study.

Based on table 2 above, the results of the analysis show a change in knowledge improvement between before and after being given education using leaflet media. This increase in knowledge can be seen from the results of the average pretest value of 19.55 ± 291 and posttest of 22.05 ± 2.04 . Providing education using leaflet media also shows results in the form of a p-value of <0.001 , meaning that there is an effect of providing education about anemia and nutrition using leaflet media on the knowledge of young women. The results of this study are in accordance with research conducted by Puspikawati et al. (2021) which showed differences in anemia knowledge before and after socialization.

Leaflets were chosen as one of the media used because leaflets can be used in all age groups, are easy to carry, see and understand, can be learned by yourself, and can provide more detailed information than being told verbally. The information conveyed in the leaflet, namely the definition of anemia, why young women are more susceptible to anemia, causes, consequences, signs, and prevention of anemia and substances that should not or need to be reduced in consumption to prevent anemia. This information was provided to increase the knowledge of young women about anemia and nutrition. Adequate knowledge about anemia and nutrition will help young women to familiarize themselves with healthier attitudes and behaviors. Research by Agustina and Permatasari (2019) shows in the results of their research that a sufficient level of knowledge can affect our attitudes and actions in choosing healthy foods, regulating consumption

patterns and the types of foods recommended to prevent anemia. After respondents studied the leaflet given, most respondents could understand anemia and nutrition better.

One of the programs to overcome and prevent anemia is the provision of supplementation of blood supplement (TTD) which is distributed to schools. However, due to the low knowledge of anemia and nutrition among young women, many do not take the supplement. Therefore, this information needs to be included in the provision of education using leaflets so that young women understand better.

The Effect of Providing Animated Video Education Media on Anemia and Nutrition on Knowledge in Young Women

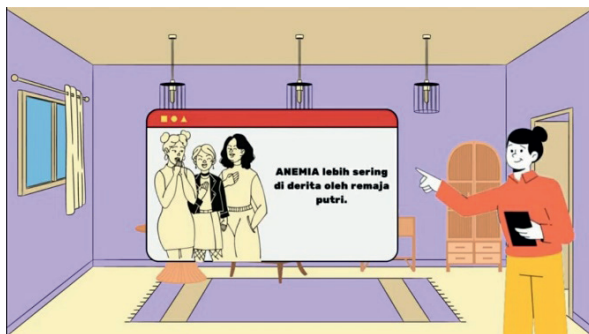


Figure 2. Animated Video on Anemia and Nutrition

Before being given education using animated video media, 20 young women respondents filled out the pretest first. After being given education using animated video media, respondents will again be asked to fill out a posttest. The results of the pretest and posttest will then be analyzed using the paired t-test.

Changes in the increase in knowledge obtained by respondents before and after being given education using animated video media can be seen in Table 2. This increase in knowledge is stated after seeing the results of the p-value of 0.017 (<0.05) so that it can also be stated that providing education using animated video media about anemia and nutrition has a significant effect on the knowledge of adolescent girls. The results of this analysis are in accordance with research conducted by Muyassaroh and Isharyanti (2020) that the provision of health education using video media provides an increase in knowledge in adolescents about premarital anemia.

Animated video is one type of audio-visual media that contains sound and image elements in it. Animated videos used in educational activities are considered to help respondents better understand information, because participants can use two senses directly rather than just looking at information in a book or listening to an explanation of the information (Puspitasari & Satriyandari, 2018).

Providing education using video provides an increase because respondents will more easily understand the information provided by using the senses of sight and hearing than using only one of the senses. In addition, video media has several advantages compared to other media, including videos that can be viewed repeatedly and explanations provided in videos accompanied by images or several properties that can describe the information described so that it is easier for respondents to understand. This is supported by a statement stating that health education using video media can improve learning quite high, because it uses the five senses of vision and hearing (Muyassaroh & Isharyanti, 2020).

Effect of QuizWhizzer Game Education Media on Anemia and Nutrition on Knowledge in Adolescent Girls

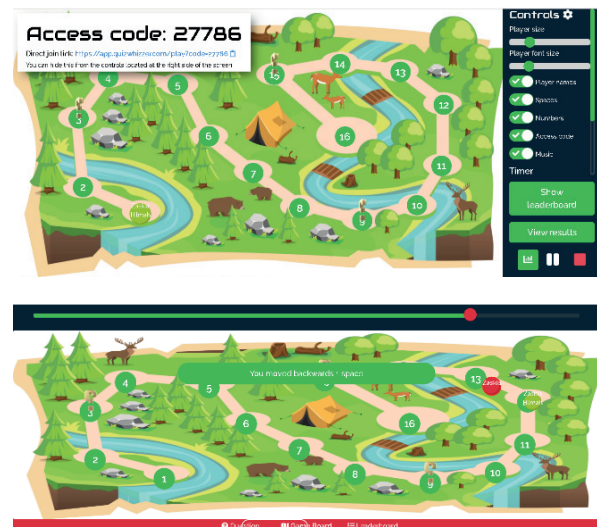


Figure 3 QuizWhizzer Game on Anemia and Nutrition

Data analysis used paired t-test to see the increase in knowledge of adolescent girls about anemia and nutrition using quizwhizzer game media. This game is an online game that is done individually and participated by 20 respondents.

Before playing this game, respondents will be instructed to fill out a pretest first, after which respondents will play while understanding the education provided in this game. Then, after playing and understanding the education about anemia and nutrition, respondents will fill out the posttest.

Average knowledge before and after education using quizwhizzer media. The average knowledge before being given education using quizwhizzer media is known to be 20.85 ± 2.37 . The average knowledge after being given quizwhizzer media is 21.90. Knowledge increased after being given education with this game media, indicating a change in the knowledge of respondents. The p-value obtained was 0.012 ($P < 0.05$) which showed that there was an effect of education about anemia and nutrition with quizwhizzer media on the knowledge of adolescent girls. The results of this study are in accordance with research Titisari and Subagyo (2019) which shows the results that the use of game media as a tool to convey anemia knowledge at SDIT Al Falah Sambu has an influence and tends to increase anemia knowledge.

Education provided with game media can help respondents to better understand the information conveyed, respondents will become more interested and relaxed in learning or paying attention to the information in educational games. The use of games as an educational tool is something interesting because students can learn while playing so that it does not cause boredom in students. (Alfiah et al., 2021).

Mean Difference in Knowledge Before and After Education

Table 2. Mean Differences in Knowledge Before and After Education

Educational Media	Average \pm SD		p-value
	Pretest	Posttest	
Leaflet	19.55 \pm 2.91	22.05 \pm 2.04	0.000
Animation Video	19.89 \pm 3.38	22.0 \pm 3.70	0.017
QuizWhizzer	20.85 \pm 2.37	21.90 \pm 1.45	0.012

Table 2 shows the differences in knowledge on the three media before and after education. Based

on the contents of Table 2, it is known that each media has an influence on increasing respondents' knowledge. The increase in knowledge among respondents in the leaflet media group was higher than those in the animated video and quizwhizzer media groups. This is likely due to the leaflet's simpler appearance, attractive colors, and easier to understand. The average educational media group with the least visible increase in knowledge is the animated video media group. The lack of visible increase in knowledge among respondents in the animated video group may be due to respondents feeling bored and sleepy while watching the animated video because the song used as the background of the animated video tends to be calm or soft.

CONCLUSIONS AND SUGGESTIONS

Providing education about anemia and nutrition using leaflets, animated videos, and quizwhizzer media has an effect on increasing knowledge in adolescent girls in Bekasi city. The highest increase in knowledge is found in leaflet media. The use of leaflet media, animated videos, and QuizWhizzer online games as tools in providing education about anemia and nutrition can increase the knowledge of adolescent girls.

In future studies, anemia education programs can be developed in junior high schools (SMP) and senior high schools (SMA) by considering this study, which directly explains to adolescent girls with the help of leaflet media that contains a brief explanation of anemia so that it is easier to understand. In addition, if using video or other audio-visual media, make sure to explain interestingly, not long-winded, and clearly. If using game media, especially online games, it should be noted that all respondents already understand the procedures for implementing the game and of course look for games that are simple and interesting but can provide appropriate education so that respondents do not feel difficult or bored while playing the educational game.

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A NARRATIVE REVIEW OF KNOWN PLANTS WHICH HAVE POTENTIAL BENEFITS AS NATURAL GALACTAGOGUES IN INDONESIA

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ABSTRACT

Human milk is the gold standard for infant nutrition during the first six months of life. According to Indonesia Demographic and Health Survey in 2017, about 45% of mothers in Indonesia provided pre-lacteal feeding within three days postpartum. Low or no milk production and perceived insufficient milk for the infants need were common reasons for pre-lacteal feeding. Galactagogues are believed to help augment human milk production. Therefore, galactagogue consumption is highly recommended, especially natural galactagogue, to help overcome low milk production problem. Most natural galactagogues are used as a local tradition only. Scientific studies regarding the phytochemical compounds and the mechanisms involved are still very limited. This narrative review aimed to describe selected research articles reporting the potency of torbangun, katuk, kelor, and papaya leaves as known plants acting as natural galactagogues in Indonesia. A total of 22 original research articles from Indonesia written in Indonesian or English over a 20 years period were included in this review. Studies were selected if selected natural galactagogues were evaluated at least for the proposed mechanism of action and effects. The data have shown strong evidence about the positive effect of torbangun, katuk, kelor, and papaya leaves as natural galactagogues on increasing human milk production. The five leaves contain different phytoestrogens that have an estrogen effect to stimulate prolactin production and increase milk production. Further studies at the molecular level are needed to identify underlying mechanisms contributing to the effectiveness and safety of the five leaves as natural galactagogues to make it easier to extend into generalizable findings. Individual variability must also be concerned because it may cause different responses to the galactagogues given.

Keywords: natural galactagogue, torbangun, katuk, kelor, papaya, milk production

INTRODUCTION

Good nutrition is essential for the survival, physical growth, mental development, productivity, health, and well-being of infants (WHO, 2000). Optimal nutrition during the first 1000 days of life will provide short and long-term health outcomes, including lowering morbidity and mortality, reducing the risk of chronic diseases, and enhancing better development (WHO, 2020). Breastfeeding has an important role, at least through exclusive breastfeeding, in providing 100% of the nutrition the infants need before six months.

Human milk is the gold standard for infant nutrition during the first six months of life. It contains all macronutrients (carbohydrate, fat, and protein) and micronutrients (vitamins and minerals) the newborns need. It also contains

bioactive molecules (immunoglobulin, growth factor, hormone) that are important for the immune system, organ development, microbial colonization in the digestive tract, and protecting infants from infection and inflammation (Ballard and Morrow, 2013).

Human milk compositions depend on maternal diet and body stores (Valentine and Wagner, 2013). Lactating mothers should maintain human milk quality and quantity to meet the balanced nutrition the infants need. Lactation, the process of human milk production and secretion, is affected by two lactogenic hormones, prolactin and oxytocin. They work independently at different cell receptors, but the combination of both is very important for successful lactation (Johnston and Amico, 1986). So, human milk production is a complex process involving physical and psychological factors and hormone interactions (Brodrigg, 2018).

Galactagogues are believed to help initiate, maintain, and augment human milk production (Brodribb, 2018). Galactagogue can be synthetically made or naturally derived from plants. Synthetic galactagogues, such as chlorpromazine, sulpiride, metoclopramide, and domperidone, are commonly used but may cause side effects to mothers such as dry mouth, indigestion, insomnia, irregular heart rhythm, hypertension, tremors, sudden death, and to infants such as indigestion and loss of consciousness (Zuppa and Sindico, 2010). Therefore, using natural galactagogue is highly recommended.

Most of the natural galactagogues are used as a local tradition only. The effects have been studied in animals and humans, but scientific studies regarding the phytochemical compounds and the mechanisms involved are still very limited (Tabares et al., 2014). Some studies mentioned that natural galactagogues contain various phytochemical components, such as alkaloids, isoflavones, polyphenols, tannins, and saponins, that may act as phytoestrogens with estrogen effect to stimulate prolactin production and increase milk production (Mohanty et al., 2014).

Only 44% of infants worldwide were exclusively breastfed (WHO, 2020). Meanwhile, in the Sustainable Development Goals agenda, World Health Organization and United Nations International Children's Emergency Fund target exclusive breastfeeding coverage to reach a minimum of 50% by 2025 (Davidove and Dorsey, 2019). In 2020, exclusive breastfeeding coverage in Indonesia was 66.06% (Kemenkes, 2021), exceeding the Strategic Plan target of 60% by 2024 (Kemenkes, 2020). However, Indonesia Demographic and Health Survey in 2017 showed that 45% of mothers in Indonesia provided pre-lacteal feeding (Rahmartani et al., 2020). Pre-lacteal feeds are any foods or drinks other than human milk given to newborns before breastfeeding initiation or milk comes in, usually within three days postpartum (Chea and Asefa, 2018). It turned out that low or no milk production and perceived insufficient milk have become common reasons for pre-lacteal feeding (Nandini et al., 2017; Odom et al., 2013; Robert et al., 2014). To help overcome this problem, besides nonpharmacological interventions, natural galactagogue consumption is

highly recommended (Brodribb, 2018) to improve milk production.

According to Widayanti (2015), katuk (*Sauropus androgynous* (L). Merr), torbangun (*Coleus amboinicus* Lour.), papaya (*Carica papaya* Linn.), kelor (*Moringa oleifera* Lam.), bitter gourd (*Momordica charantia*), and mungbean (*Vigna radiate*), are some plants that are commonly used in Indonesia as natural galactagogues. Three plants listed as natural galactagogues in the Regulation of the Minister of Health of the Republic of Indonesia no. 6/2016 regarding Indonesian native drug formulary are torbangun, katuk, and fenugreek (Kemenkes, 2016). This literature review aimed to describe selected research articles reporting the potency and efficacy of selected known plant leaves which believed to have potential benefits as natural galactagogues in Indonesia.

METHODS

A literature review was conducted by searching published and gray literature related to known plants traditionally used as natural galactagogues in Indonesia. The online search from IPB University Scientific Repository, Google Scholar, and Google Website was conducted using the following keywords either in solo or in combination: galactagogue, natural galactagogue, Indonesia, human milk, breast milk, phytochemical, phytoestrogen, phytosteroid, torbangun, katuk, kelor, moringa, papaya, and leaves, within the articles. Original research articles from Indonesia that were published in Science and Technology Index- and Scopus-indexed journals, nonindexed journals, and gray literature which are published informally, non-commercially published, or unpublished, including theses, dissertations, and unpublished articles, were included in this review. The review was limited to articles in English and Indonesian languages with lactating mice, rats, and humans as research subjects. All types of in vivo study were included in this review to get an in-depth understanding of the efficacy of selected known natural galactagogues commonly used in Indonesia. Studies were selected if natural galactagogues were evaluated, at least for the proposed mechanism of action and effects. With the consideration of language, in vivo study types,

and details of the study result required for this review, articles written over a 20 years period were included.

RESULTS AND DISCUSSION

Table 1 summarized several studies about the potency of torbangun (Damanik et al., 2017), katuk (Handayani et al., 2020; Soka et al., 2011; Subekti, 2007; Suprayogi et al., 2015), fenugreek (Bumrungpert et al., 2018; Gabay, 2002; Turkyilmaz et al., 2011), kelor (K et al., 2013; King et al., 2013; Setiasih et al., 2019; Utary et al., 2019), and papaya (Canini et al., 2007; Ikhlasiah et al., 2020; Setyono et al., 2016; Sugiyanto et

al., 2012) as five known natural galactagogues in Indonesia. The part used as a galactagogue for fenugreek is the seeds, while torbangun, katuk, kelor, and papaya are the leaves. Based on previous studies, these parts have an estrogen effect to help increase milk production. Torbangun, katuk, kelor, and papaya were selected for this review as they use the leaves as natural galactagogues. A total of 22 articles representing in vivo studies of these plants as natural galactagogues were included in this review. Three studies were using lactating mice, nine studies were using lactating rats, and 10 studies were using lactating mothers.

Table 1. Some of the known plants that are believed as natural galactagogues in Indonesia






Plant name	Picture	Part	Proposed effect/mechanism	Phytochemical components and active compounds predicted to act as a galactagogue	Reference
Torbangun (<i>Coleus amboinicus</i> Lour.)		Leaf	Estrogen effect (increase proliferation in mammary epithelial cells, induce prolactin receptor expression (PRLR), increase casein production, and increase lactose synthetase activity in mammary epithelial cells to increase milk production)	Phenol (digiprolactone, kaempferol-3,7-O-di-ramnopyranoside (kaempferitin))	Damanik et al., 2017
Katuk (<i>Sauropus androgynus</i> (L). Merr)		Leaf	Estrogen effect, vasodilator	Sterol (stigmasterol, sitosterol, fucosterol, androstan-17-one, 3-ethyl-3-hydroxy-5-alpha), alkaloid (papaverine)	Handayani et al., 2020; Soka et al., 2011; Subekti, 2007; Suprayogi et al., 2015
Fenugreek (<i>Trigonella foenum-graecum</i> Linn.)		Seed	Estrogen effect, increase sweat production so that it is predicted to increase milk production because mammary glands are modified sweat glands	Sapogenin steroid (diosgenin), flavon (apigenin, luteolin)	Bumrungpert et al., 2018; Gabay, 2002; Turkyilmaz et al., 2011
Kelor (<i>Moringa oleifera</i> Lam.)		Leaf	Estrogen effect	Sterol (campesterol, stigmasterol, β-sitosterol), isoflavone (daidzein, biochanin A, glycitein)	K et al., 2013; King et al.; 2013; Setiasih et al., 2019; Utary et al., 2019
Papaya (<i>Carica papaya</i> Linn.)		Leaf	Estrogen effect	Flavonol (kaempferol, quercetin)	Canini et al., 2007; Ikhlasiah et al., 2020; Setyono et al., 2016; Sugiyanto et al., 2012

Table 2 summarized the characteristics of torbangun, katuk, kelor, and papaya leaves in the edible part of fresh, simplicia, and extract. The comparison of the four fresh leaves is as follows (Mahmud et al., 2018). Kelor leaves have the highest energy, carbohydrates, fiber, ash, vitamin

Table 2. Characteristics of torbangun, katuk, kelor, and papaya leaves

Parameter	Torbangun leaves	Katuk leaves	Kelor leaves	Papaya leaves
	Content in 100 g edible part of fresh leaves (Mahmud et al., 2018) / dry leaves (Iwansyah, 2018)			
Protein (g)	1.3 / 17.36	6.4 / 29.46	5.1 / 25.22	8 / 30.85
Fat (g)	0.6 / 5.87	1 / 6.73	1.6 / 5.67	2 / 5.46
Carbohydrate (g)	4 / 52.18	9.9 / 45.09	14.3 / 48.91	11.9 / 45.77
Fiber (g)	1 / 11.13	1.5 / 9.86	8.2 / 9.34	1.5 / 10.62
Water (g)	92.5 / 10.33	81 / 9.03	75.5 / 9.24	75.4 / 7.70
Ash (g)	1.6 / 14.26	1.7 / 9.71	3.5 / 10.97	2.7 / 10.28
Content in 100 g edible part of fresh leaves (Mahmud et al., 2018)				
Vitamin				
β-carotene (µg)	1,489	9,152	3,266	5,409
Total carotene (µg)	13,288	10,020	NA	18,250
Thiamine (vitamin B1) (mg)	0.16	0	0.3	0.15
Riboflavin (vitamin B2) (mg)	0.1	0.31	0.1	0.5
Niacin (vitamin B3) (mg)	0	2.3	4.2	1.9
Vitamin C (mg)	5	164	22	140
Mineral				
Calcium (mg)	279	233	1,077	353
Phosphor (mg)	40	98	76	63
Iron(mg)	13.6	3.5	6	0.8
Zinc (mg)	NA	1.3	0.6	1.5
Kalium (mg)	144	478.8	298	926.6
Sodium (mg)	4	21	61	4
Copper (mg)	NA	0.3	0.1	0.2
Simplicia (Iwansyah, 2018)				
pH	7.05	6.46	5.74	6.05
Aw	0.67	0.44	0.47	0.51
Color	dark, slightly reddish, slightly yellow	dark, slightly greenish, more yellow	dark, slightly greenish, more yellow	dark, slightly greenish, more yellow
L (lightness)	33.67	42.06	35.73	34.33
a (redness)	4.58	-4.51	-4.81	-3.04
b (yellowness)	10.81	23.37	22.58	20.88
95% ethanol extract (Iwansyah, 2018)				
Yield (%)	0.85	2	0.95	1.25
Phytochemical component				
Total phenol (mg GAE/g)	49.98	27.49	53.89	47,78
Total flavonoid (mg QE/g)	14.49	43.63	36.60	21,73
70% ethanol extract (Subekti, 2007) Extract (Kristina and Syahid, 2014)				
NA 1.1 (stigmasterol), 0.69 (sitosterol), 0.64 (fukosterol) 1.15 (sitosterol), 1.52 (stigmasterol) NA				
Total sterol (%)	NA			NA

NA = not available; GAE = mg equivalent gallic acid; QE = mg equivalent quercetin

B1, vitamin B3, mineral Ca, and Na content. Papaya leaves have the highest protein, fat, total carotene, vitamin B2, mineral Zn and K content. Katuk leaves have the highest β -carotene, vitamin C, mineral P, and Cu content. Torbangun leaves have the highest mineral Fe content. Consecutively, kelor and katuk leaves 95% ethanol extracts have the highest total phenolic and flavonoid content compared to others (Iwansyah, 2018). Sterol content is found in katuk (Subekti, 2007) and kelor leaf extracts (Kristina and Syahid, 2014).

Torbangun leaf

Torbangun leaves contain phenol components (digiprolactone and kaempferol-3,7-O-dicamnopyranosida (kaempferitin)) in torbangun leaves ethyl acetate fraction. They work synergistically and are predicted to play a role in stimulating and increasing milk production (Damanik et al., 2017). Torbangun leaves also contain alkaloid, flavonoid, and tannin and are listed as a grade C natural galactagogue in the Regulation of the Minister of Health of the Republic of Indonesia no. 6/2016 regarding Indonesian native drug formulary based on previous preclinical studies to goats and cows and clinical study to lactating mothers aged 20 to 40 years old, who gave normal birth to babies with minimum birth weight 2.5 kg and gave exclusive breastfeeding for a minimum of four months. The suggested dosage for lactating mothers was 3x50 g torbangun simplicia per day, and the LD₅₀ (50% lethal dose) was more than 5000 mg/kg of body weight in the rat (Kemenkes, 2016). Table 3 summarized selected in vivo studies of torbangun leaves as a galactagogue using lactating rats (Damanik et al., 2017; Hasianna et al., 2021; Hutajulu and Junaidi 2013; Iwansyah et al., 2017) and humans (Herlina, 2018; Santosa, 2001).

Hasianna et al. (2021) showed that 432 mg of dried torbangun leaves had the most significant effect on increasing prolactin level compared to the negative control, 108 mg, and 216 mg of dried torbangun leaves. There was no significant difference in the weight gain of the pups. Consecutively, the weight gain of the pups from the highest to the lowest was in 432 mg, 216 mg, 108 mg of dried torbangun leaves, positive control (1 mg of domperidone), and negative control

treatment. Damanik et al. (2017) showed that milk production from the highest to the lowest was in 50 mg/kg of kaempferol, 50 mg/kg of commercial human milk booster (containing 114 mg of katuk leaves extract, 20 μ g of vitamin B12, 25 mg of vitamin B2, 10 mg of vitamin B1), 30 mg/kg torbangun leaves ethyl acetate fraction, control, and 80 mg/kg torbangun leaves aqueous extract treatment. Iwansyah et al. (2017) also showed that the weight gain of the pups in torbangun leaves ethyl acetate fraction treatment was significantly different from the control, and the average weight gain of the pups in torbangun leaves ethyl acetate fraction treatment was higher compared to the commercial booster and control. In addition, there was no significant difference in the weight gain of the lactating rats. Hutajulu and Junaidi (2013) also showed that torbangun leaves extract could increase milk production as indicated by the higher weight gain of the pups compared to the control.

Herlina et al. (2018) showed that consumption of 500 mg of torbangun leaves extract three times a day in lactating mothers could significantly increase the prolactin level of the mothers, weight of the babies, urination and defecation frequency of the babies, sleep duration of mothers and babies, and frequency of the mothers to breastfeed, compared to control. Santosa (2001) showed the consumption of 150 g of fresh torbangun leaves every two days could significantly increase milk production, the weight of the babies, and the iron content in human milk. In addition, there was also a significant increase in the potassium content of human milk compared to the commercial human milk booster (Lancar ASI and Moloco) and in the zinc content of human milk compared to the commercial human milk booster (Moloco). Although not significantly different, there was also an increase in the protein, calcium, and magnesium content of human milk and the prolactin level of the mothers.

Katuk leaf

Katuk leaves contain sterol components (stigmasterol, sitosterol, fucosterol (Subekti, 2007), androstane-17-one, 3-ethyl-3-hydroxy-5- α (Suprayogi et al., 2015)) and alkaloid component (papaverine) (Soka et al., 2010). Both have a positive effect as phytoestrogens to

Table 3. Several studies about the efficacy of torbangun leaves as a natural galactagogue in Indonesia

Subject and period	Dosage	Result	Reference
15 Sprague-dawley rats, weight range between 250 to 300 g, for 13 days (from day two to day 14 after delivery)	Once every two days: 30 mg/kg of torbangun leaves ethyl acetate fraction 50 mg/kg of commercial human milk booster (containing 114 mg of katuk leaves extract, 20 µg of vitamin B12, 25 mg of vitamin B2, 10 mg of vitamin B1 Control (aquadest)	Semipolar compounds group in torbangun leaves ethyl acetate fraction can be considered as a trigger for milk production as indicated by a significantly higher total milk production yield in torbangun leaves ethyl acetate fraction compared to the control. There was no significant difference in the weight gain of the lactating rats. The weight gain of the pups treated with torbangun leaves ethyl acetate fraction was significantly higher compared to the control.	Iwansyah et al., 2017
Rats, for 14 days (from 7 days before delivery to 7 days after delivery)	-Fresh torbangun leaves extract (25 kg of fresh leaves in 500 ml hot water) -Dry torbangun leaves extract (3%, 5%, 7% of dry leaves in 500 ml hot water) -Control	-Torbangun leaves extract consumption can increase milk production as indicated by a weight gain of the mother and pups in fresh torbangun leaves extract treatment. Meanwhile, in other treatment, there was a decrease in the weight of lactating rats, with the highest reduction in the control and the lowest reduction in the dry torbangun leaves extract treatment. -The highest weight gain of the pups was in 5% dry torbangun leaves extract treatment compared to the other extracts. The lowest weight gain of the pups was in the control.	Hutajulu and Junaidi, 2013

Subject and period	Dosage	Result	Reference
16 lactating Batak women, for 21 days (from day 4 to day 24 after delivery)	-150 g of fresh torbangun leaves every two days (in the form of a food menu) -“Kaptab” “Lancar ASI” three times a day (containing 25% of katuk leaves extract) Moloco + vitamin B12 pills three times a day (containing 15 mg of placenta extract and 20 mg of vitamin B12) -Control (only daily food menu)	The effect of torbangun leaves treatment compared to the control and other treatments: -Significant increase in milk production. -Significant increase in weight of the babies but no significant difference in the body length and head circumference of the babies. -Significant increase in the iron content of human milk. -An increase in the potassium content of human milk with a significant difference only with the “Lancar ASI” and Moloco Pills treatment only. -Increased zinc content of human milk with a significant difference only with (Moloco + vitamin B12) treatment. -Increased level of protein, calcium and magnesium although there was no significant difference. Higher level of prolactin although there was no significant difference.	Santosa,2001
30 lactating Wistar rats, for 14 days	Three times a day: 108 mg of dry torbangun leaves 216 mg of dry torbangun leaves 432 mg of dry torbangun leaves Positive control (1 mg of domperidone) Negative control (10% of CMC)	-All dried torbangun leaves treatments gave the effect to increase prolactin level compared to the negative control. -Treatment of 432 mg of dried torbangun leaves gave the most significant effect to increase prolactin level compared to the negative control, 108 mg, and 216 mg of dried torbangun leaves. -No significant difference in prolactin level of 108 mg and 216 mg of dried torbangun leaves compared to the negative control. -No significant difference in the weight gain of the pups between treatments.	Hasianna et al., 2021

Subject and period	Dosage	Result	Reference
32 lactating mothers after delivery, for 14 days	500 mg of torbangun leaves extract, three times a day Control	The effect of torbangun leaves extract treatment compared to control: -Significant increase in the prolactin level of the mothers, weight, urination dan defecation frequency of the babies. -Significant increase in the duration of sleep of the mothers and babies. -Significant increase in the frequency to breastfeed.	Herlina et al., 2018
25 lactating rats, weight range between 250 to 300 g, for 27 days (from day two to day 28 after delivery	Once every two days: -30 mg/kg of torbangun leaves ethyl acetate fraction -80 mg/kg of torbangun leaves water extract -60 mg/kg of kaempferol -50 mg/kg of commercial human milk booster (containing 114 mg of katuk leaves extract, 20 µg of vitamin B12, 25 mg of vitamin B2, 10 mg of vitamin B1) -Control	-Consecutively, the increase in milk production from the highest to the lowest was in kaempferol, commercial human milk booster, torbangun leaves ethyl acetate fraction, control, and torbangun leaves aqueous extract treatment. -Torbangun leaves ethyl acetate fraction treatment did not show any toxicity symptoms in mammary the gland, liver, intestines, and kidney. -The content of digiprolactone and kaempferol derivatives in ethyl acetate fraction is predicted to play a role in stimulating and increasing milk production.	Damanik et al., 2017

induce prolactin and oxytocin gene expression. Papaverine is a vasodilator that may increase blood vessel dilation to facilitate prolactin and oxytocin circulation through blood vessels (Handayani et al., 2020). The hexane extract contains an aliphatic compound. The ether extract contains monomethyl succinate benzoic acid, and 2-phenylmalonic acid as major compounds, and terbutol, 2-propagytoxane, 4H-pyran-4-one, 2-methoxy-6-methyl-, 3-penten-2-one, 3-(2-furanyl)-, and palmitic acid as minor compounds. The ethyl acetate extract contains cis-2-methyl-cyclopentanol acetate, 2-pyrrolidinone, and methyl pyroglutamate as the major compounds and p-dodecyl phenol (Agustal et al., 1997), protein, fat, calcium, phosphorus, iron, vitamins A, B, and C as the minor compounds (Kemenkes, 2016). Katuk leaves are listed as a natural galactagogue

in the Regulation of the Minister of Health of the Republic of Indonesia no. 6/2016 regarding Indonesian native drug formulary based on previous preclinical studies to rats and lambs and clinical study to lactating mothers. The suggested dosage for lactating mothers was 3x300 mg extract daily, and the LD₅₀ in rats was more than 5000 mg/kg of body weight. 20%, 40%, and 80% of leaves infusion to mice during organogenesis didn't show any teratogenic signs (Kemenkes, 2016). Table 4 summarized selected in vivo studies of katuk leaves as a galactagogue using lactating rats (Miharti et al., 2018; Suprayogi, 2015), mice (Darsono et al., 2014; Soka et al., 2010; Soka et al., 2011) and humans (Nasution, 2018; Sa'roni et al., 2004; Situmorang and Singarimbun, 2019). Miharti et al. (2018) showed that 72 mg of katuk extract effectively increased prolactin

level in lactating rats because it could stimulate prolactin receptors in lactotroph cells to trigger neurohormones in stimulating the release of Prolactin Releasing Factor. Suprayogi et al. (2015) showed that katuk leaves hexane fraction contains sterol components (androstane-17-one, 3-ethyl-3-hydroxy-5- α) which can increase milk production by stimulating estrogen. It was demonstrated that milk production in lactating rats treated with 57.5 mg/day of hexane fraction increased compared to the control. Milk production

in lactating rats treated with 40 mg/day of ethyl acetate fraction and 297.5 mg/day of crude ethanol extract was lower, while 209 mg/day of aqueous fraction gave the same milk production compared to the control. In addition, the average weight gain of the pups in lactating rats treated with hexane fraction significantly increased compared to the control.

Darsono et al. (2014) showed that consecutively, the most effective treatment to increase the number of mammary alveoli in

Table 4. Several studies about the efficacy of *katuk* leaves as a natural galactagogue in Indonesia

Subject and period	Dosage	Result	Reference
80 pregnant rats, for 24 days (from day 8 to day 21 of pregnancy, and the treatment was continued after delivery until day 10 of lactation)	-57.5 mg/day of hexane fraction of katuk leaves extract (19.3% of crude ethanol extract) -40 mg/day of ethyl acetate fraction of katuk leaves extract (13.4% of crude ethanol extract) -209 mg/day of water fraction of katuk leaves extract (70% of crude ethanol extract) -297.5 mg/day of katuk leaves crude ethanol extract -Control	-Hexane fraction treatment showed an increase in milk production as indicated by a significant increase in the average weight gain of the pups, compared to the control. The hexane fraction contains sterol components (androstan-17-one and 3-ethyl-3-hydroxy-5- α) which can increase milk production by stimulating estrogen. -Milk production in ethyl acetate fraction and crude ethanol extract treatment was lower while water fraction treatment gave the same milk production compared to the control.	Suprayogi et al., 2015
30 female mice after delivery, for 12 days	-173.6 mg/kg/day of katuk leaves extract -868 mg/kg/day of katuk leaves extract -Mixture of 173.6 mg/kg/day of katuk leaves extract and 0.078 mg/day of domperidone -Positive control (Moloco) -Negative control	-Consecutively, the most effective treatment in increasing the number of mammary alveoli was the positive control, 173.6 mg/kg of the extract, and a mixture of katuk leaves extract and domperidone. -There was a significant difference between the negative control and positive control, 173.6 mg/kg of extract, and the mixture of katuk leaves extract and domperidone treatment.	Darsono et al., 2014
24 lactating BALB/C mice, for 12 days	Two times a day: -173.6 mg/kg/day of mature katuk leaves extract -868 mg/kg/day of mature katuk leaves extract -Control	-There was a decrease in prolactin gene expression in higher concentration of katuk leaves extract treatment compared to negative control because prolactin secretion will increase dopamine secretion which can inhibit prolactin secretion (mechanism of short-loop feedback). -There was an increase in oxytocin gene expression in higher concentration of katuk leaves extract treatment because papaverine content in katuk leaves which acts as a vasodilator to facilitate oxytocin circulation through blood vessels.	Soka et al., 2011

Subject and period	Dosage	Result	Reference
24 lactating BALB/C mice, for 12 days	-173.6 mg/kg/day of young katuk leaves extract -173.6 mg/kg/day of mature katuk leaves extract -Control	The content of papaverine in mature katuk leaves (0.38 ± 0.04 µg/ml) induced prolactin and oxytocin gene expression as indicated by the increase in prolactin and oxytocin gene expression in mature leaves extract (consecutively 15.75 and 25.77 times) was higher than in the young leaves extract (consecutively 9.04 and 2.25 times).	Soka et al., 2010
96 lactating mothers, for 15 days	Three times a day: -300 mg of katuk leaves extract -Control	-Katuk leaves extract treatment gave a significant effect on increasing human milk production by up to 50.7%.	Sa’roni et al., 2004
32 lactating mothers, for 10 days	Three times a day: -150 ml of boiled katuk leaves (300 g of katuk leaves boiled with 1.5 L of water) -Control	There was a significant increase in milk production of boiled katuk leaves treatment compared to the control.	Situmorang and Singarimbun, 2018
24 lactating Wistar rats, weight range between 150 to 200 g, for 8 days	-24 mg/day of katuk leaves ethanol extract -48 mg/day of katuk leaves ethanol extract -72 mg/day of katuk leaves ethanol extract -Control	-The higher the ethanol extract, the higher increase in prolactin production of lactating rats because it could stimulate prolactin receptors in lactotropic cells as prolactin producers to trigger neuro hormone which will stimulate the release of Prolactin Releasing Factor (PRF). -There was a significant difference in the increase in prolactin production between the control and 72 mg extract treatment, and between 24 mg and 72 mg extract treatment.	Miharti et al., 2018
24 mothers after delivery, for 2 weeks	Two times a day: -2 g of dried katuk leaves tea -Control	There was a weight gain of the babies and significant increase in milk production of katuk leaves tea treatment compared to the control.	Nasution, 2018

lactating mice was in commercial human milk booster (Moloco), 173.6 mg/kg of katuk leaves extract, and a mixture of 173.6 mg/kg/day of katuk leaves extract and 0.078 mg/day of domperidone. Soka et al. (2011) showed that lactating mice treated with 173.6 mg/kg of mature katuk leaves extract had the most effective effect on prolactin and oxytocin gene expression, followed by 868 mg/kg of extract compared to the control. Prolactin secretion may increase dopamine secretion, inhibiting prolactin secretion, the so-called short-loop feedback mechanism. In higher katuk leaves extract treatment, prolactin gene expression decreased.

On the other hand, papaverine content in katuk leaves may inhibit dopamine receptors so that it may restimulate prolactin secretion. The increase in oxytocin gene expression occurred due to papaverine content in katuk leaves which acts as a vasodilator. Soka et al. (2010) showed that the increase in prolactin and oxytocin gene expression in lactating mice occurred in mature katuk leaves

extract higher than in young katuk leaves extract treatment.

Situmorang and Singarimbun (2019) showed that milk production and defecation, urination, and weight of the babies of lactating mothers treated with water-boiled katuk leaves significantly increased compared to the control. Nasution (2018) also showed that the milk production and weight of the babies of lactating mothers treated with katuk leaves tea significantly increased compared to the control. Sa’roni et al. (2004) showed that milk production of lactating mothers treated with 300 mg of katuk leaves extracts three times a day significantly increased by up to 50.7% compared to the control.

Kelor leaf

Kelor leaves contain sterol components (campesterol, stigmasterol, β-sitosterol (K et al., 2013)) and isoflavone components (daidzein, biochanin A, glycitein (Setiasih et al., 2019)) which have a positive effect as phytoestrogens.

Table 5 summarized selected in vivo studies of kelor leaves as a galactagogue using lactating rats (K et al., 2013; Utary et al., 2019) and humans (Renityas, 2018; Sulistiawati et al., 2017).

Utary et al. (2019) showed that kelor leaves ethanol extract positively affected the alveoli growth of lactating rats, depending on the given dosage. Treatment of 168 mg/kg of extract gave a better effect than 42 mg/kg. Although not significantly different, the weight gain of the pups in ethanol extract treatment was higher than in the control. K et al. (2013) showed that the birth weight of the pups and after 15 days after birth was highest in lactating rats treated with 168 mg/kg dried steam-blanchd kelor leaves treatment and lowest in the control. Phytosteroids in kelor leaves act as precursors of steroid hormones like

estrogen. Renityas (2018) showed that 800 mg of kelor leaf extract capsules treatment significantly increased the milk production of lactating mothers. Sulistiawati et al. (2017) showed that 250 mg kelor leaves capsules treatment significantly increased the prolactin level of the mothers and sleep duration of the babies. Although not significantly different, the weight gain of the babies was higher in 250 mg kelor leaves capsules treatment than in the control.

Papaya leaf

Scientific research using animals and humans regarding the efficacy of papaya leaves as a galactagogue has not been carried out much compared to the three leaves above. However,

Table 5. Several studies about the efficacy of kelor leaves as a natural galactagogue in Indonesia

Subject and period	Dosage	Result	Reference
24 pregnant female rats, aged between 8 to 10 weeks, weight between 150 to 210 g, from day 14 of pregnancy to day 7 after delivery	-42 mg/kg of dried kelor leaves ethanol extract -168 mg/kg of dried kelor leaves ethanol extract -13 mg/kg of positive control (Moloco) -Negative control (aquadest)	-Higher ethanol extract of kelor leaves gave a greater effect on the growth of mammary gland alveoli, -Higher increase in the weight of the pups in the ethanol extract compared to the control treatment. When given to pregnant woman, it is expected that kelor leaves can prevent low birth weight.	Utary et al., 2019
20 lactating mothers, for 1 month	800 mg of kelor leaves ethanol extract in capsule (leaves extract: dried leaves = 1:4)	Consumption of kelor leaves ethanol extract in the capsule was effective to significantly increase milk production of lactating mothers.	Renityas, 2018
30 mothers who were willing to give exclusive breastfeeding, for 14 days (from day 1 to day 14 after delivery)	Two times a day: -250 g of dried kelor leaves capsule -Control	The effect of kelor leaves capsule treatment compared to the control: -Significant increase in prolactin level. -Increase in weight gain of the babies. -Increase in sleep duration of the babies. Kelor leaves contain phytosteroids that have a galactagogue effect.	Sulistiawati et al., 2017
Pregnant Wistar white rats, from the beginning of pregnancy until day 14 of lactation	-42 mg/kg of dried kelor leaves -84 mg/kg of dried kelor leaves -168 mg/kg of dried kelor leaves Kelor leaves were given three treatments as follows: non-blanchd, steam-blanchd, boil-blanchd, boiled with Na ₂ CO ₃ -Control	Dried kelor leaves treatment effect: -The highest birth weight of the pups was in the rat treated with 168 mg/kg of dried steam-blanchd kelor leaves and the lowest was in the control. -After 15 days of treatment, the highest weight of the pups was in the lactating rats treated with 168 mg/kg of dried steam-blanchd kelor leaves and the lowest was in the control. Estrogen potency of kelor leaves comes from phytosteroid content such as stigmasterol, sitosterol, and kaempferol which can function as a precursor of steroid hormone like estrogen.	K et al., 2013

Table 6. Several studies about the efficacy of papaya leaves as a natural galactagogue in Indonesia

Subject and period	Dosage	Result	Reference
36 mothers after delivery, for 7 days	-458 mg/day of papaya leaves nano particle in capsule -Control	-Papaya leaves capsule treatment showed a significant increase in the weight of the babies compared to the control. -Nano particles have a very small size so that the body could absorb them easily.	Khasanah et al., 2021
24 Wistar female rats, aged between 12 to 14 weeks, weight between 200 to 225 g, for 14 days (from day 1 to day 14 after delivery)	-0.95 mg/200 g body weight/day of papaya leaves ethanol extract -1.9 mg/200 g body weight/day of papaya leaves ethanol extract -3.8 mg/200 g body weight/day of papaya leaves ethanol extract -Control (only standard meal)	-All papaya leaves ethanol extract treatments showed a significant increase in prolactin level, prolactin receptor gene expression, breast alveoli, and breast lobules compared to the control. -1.9 mg/200 mg body weight/day extract was the most effective dosage because it gave the highest increase.	Herawati et al., 2021
10 lactating mothers with babies from 0 to 6 months of age, for 7 days	Papaya leaves juice, two times a day	Papaya leaves juice treatment showed a significant increase in the weight of the babies and prolactin level of the lactating mothers compared to before treatment was given.	Ikhlasiah et al., 2020
48 mothers after delivery, for 7 days	Two times a day: -400 mg/day of papaya leaves ethanol extract powder -300 mg/day of papaya leaves ethanol extract powder -Control (only standard meal)	2x400 mg/day of extract treatment was significantly effective in increasing prolactin level and human milk production. As an indicator of human milk production, the weight of the babies was measured.	Pratiwi, 2018

papaya leaves contain flavonol components (kaempferol and quercetin), which positively affect phytoestrogens because they can activate prolactin receptors to increase milk production (Setyono et al., 2016). Table 6 summarized selected in vivo studies of papaya leaves as a galactagogue using lactating rats (Herawati et al., 2021) and humans (Ikhlasiah et al., 2020; Khasanah et al., 2021; Pratiwi, 2018).

Herawati et al. (2021) showed that papaya leaves ethanol extract significantly increased prolactin level, prolactin receptors gene expression, and the number of breast alveoli and lobules in lactating rats compared to the control. A dosage of 1.9 mg/200 g body weight/day of papaya leaves ethanol extract was the most effective treatment since it gave the highest increase. Khasanah et al. (2021) showed that 458 mg/day of papaya leaves nanoparticles in the capsule treatment

significantly increased the weight of the babies compared to the control. Nanoparticles have a very small particle size and can easily be absorbed by the body. Ikhlasiah et al. (2020) showed that after lactating mothers were treated with papaya leaves juice, there was a significant increase in the prolactin level and weight of the babies. Pratiwi (2018) showed that the consumption of 400 mg of papaya leaves ethanol extract powder twice a day significantly increased prolactin level and human milk production. The babies were weighted as the indicator of milk production.

CONCLUSION

Five plant leaves (torbangun, katuk, kelor, and papaya leaves) that have potential benefits as natural galactagogues were reviewed and showed a positive effect on increasing human

milk production. Current research on natural galactagogues is relatively inadequate to guide clinical recommendations regarding the concentration of the natural galactagogues content in each various formats of the products. Further studies at the molecular level are needed to identify underlying mechanisms contributing to the effectiveness of natural galactagogues towards the development of lactation insufficiency to make it easier to extend into generalizable findings. Evaluating the cause of low milk production, toxicity level, contradictions, warnings, side effects, and medicine interactions must also be considered before giving natural galactagogues. The evidence above is needed to facilitate the appropriate development and evaluation of novel natural galactagogues products due to individual variability that may cause different responses.

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THE EFFECT OF THE BIOACTIVE COMPOUND CURCUMIN ON CONDITIONS AFTER ISCHEMIC STROKE: A SYSTEMATIC REVIEW

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ABSTRACT

Ischemic stroke is a common degenerative disease in Indonesia caused by interrupted or restricted blood supply to part of brain, preventing it from getting oxygen and nutrients. Without sufficient blood supply, brain cells begin to die. Various treatments for ischemic stroke patients have been developed and implemented, but are still ineffective in treating or preventing brain damage. Curcumin is one of the bioactive compounds which mostly found in turmeric which is one of the main spices resource in Indonesia that has many benefits as a medicine. People have been making use of curcumin as a medicine for various diseases, one of which is stroke. Therefore, this systematic review analysed qualitatively the effect of curcumin on the brain condition after ischemic stroke. The method used in this study was a systematic review of 8 databases in the last 10 years, from 2012 to August 2022. Study included was only experimental study on rats. Based on 19 articles gathered, there was a decrement in ROS, COX-2, iNOS, NF-kB, TNF- α , IL-6, Bax, Caspase-9, Caspase-3, ICAM-1, MMP-9, neurological deficit score, and an increment in BCL-2, glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) in rats receiving curcumin intervention either orally or intravenously. Curcumin affects the ischemic brain in a number of ways, namely as an antioxidant, anti-inflammatory, anti-apoptotic, Blood-Brain-Barrier (BBB) protector, increasing neurogenesis, and reducing neurological deficits. It is concluded that curcumin has an elevating effect in protecting brain condition after an ischemic stroke.

Keywords: curcumin, ischemic stroke, brain ischemia, neurogenesis, deficit neurologic

INTRODUCTION

Stroke is a condition where an artery transporting blood to the brain ruptured or blocked, causing brain cells deprived from oxygen and died (WHO, 2020a). Stroke is the second predictor causing death and the third predictor to disability around the world (Feigin et al., 2017). Every year more than 15 million people suffering from stroke, with a third ended in death and a third ended with permanent disability (WHO, 2011). The prevalence of stroke in Indonesia increases from 7 per mill in 2013 to 10.9 per mill in 2018 (Kementrian Kesehatan, 2018).

Stroke can be divided into three categories based on the types of stroke, namely transient ischemic attack (TIA), ischemic stroke and hemorrhagic stroke (WHO, 2020a). Around 80% of strokes occurred are ischemic in nature (Krishnamurthi et al., 2013). Ischemic stroke happen when blood flow blocked by a blood clot which occur in the brain. These blood clots usually occur due to atherosclerosis or thickening

of the walls of blood vessels due to accumulated fat (WHO, 2020b). These clots then block the supply of oxygen and nutrients to brain cells, causing hypoxia, free radicals and inflammation (Jia et al., n.d.; Oh et al., 2019). Ischemic stroke leads to many complications like memory loss, motor dysfunction, fatigue, mental state change, and Alzheimer (Béjot et al., 2016). In addition, the incidence of ischemic stroke is associated with continuous treatment, inflicting unfavorable consequences in terms of socio-economic to a country (Béjot et al., 2016; Rusek & Czuczwar, 2021). Death due to stroke in young adults are currently starting to increase significantly, especially in developing countries in which the incidence of stroke is indeed common (Feigin et al., 2017).

Ischemic stroke usually treated by using thrombolysis and thrombectomy therapy which aim to restore blood flow in the brain, protect tissue and maintain brain function (Marler, 1995; Patel et al., 2020; Wang et al., 2023). However, these

treatments are limited in that their effectiveness depends on time after the event (Cassella & Jagoda, 2017). In addition, in control of free radical and oxygen level before and after thrombolysis is very crucial to save patient (Shi et al., 2020). Currently, there are no treatment that can prevent, delay/stop neurodegeneration that occur in ischemic stroke patient (Briggs et al., 2016).

Curcumin is an active compound in turmeric obtained from *Curcuma longa* Linn powder (Zhao et al., 2010). Curcumin may useful as a treatment for ischemic stroke that causes brain cell death and clinical deficits (Dirnagl et al., 1999; Lapchak & Araujo, 2007). Evidence shows that inflammation, oxidative damage and misfolded protein amyloid are the main factors of brain damage in ischemic stroke (Pluta et al., 2019; Pluta & Ułamek-Kozioł, 2021). Curcumin plays a role in 2 main key activities in stopping neural degeneration in ischemic stroke, namely free radicals production and immune system activation (Lapchak, 2010; Moskowitz et al., 2010). Research in patients in hospital show that there were no severe side effects from curcumin use but some patients develop mild diarrhea and nausea (Chh-Hung & Ann-Lii, 2007).

Curcumin is a bioactive compound with a potential to be a treatment for ischemic strokes and an interesting subject to be studied further. Thus, this paper aim to review various literatures with a narrative synthesis in interpreting some of the latest empirical literature regarding to the effect of curcumin on ischemic stroke.

METHODS

Data Sources and Article Search Methods

This study was systematic review research that would answer the question: “How is the effect of curcumin on ischemic stroke?” Search articles using 8 databases: Pubmed, Medline, Research Gate, Science Direct, Springer Link, Proquest, EbscoHost, and Google scholar. Keywords use to search articles were “Curcumin, stroke, ischemic stroke, ischemic brain, brain damage, and neuroprotection”.

Inclusion and Exclusion Criteria

The criteria for articles excluded in this study included articles with irrelevant topics, articles not available for open access, the year of publication exceeding ten years, the type of document in the form of review articles, notes or letters, and poor methodology. Articles that pass the exclusion process would be selected with inclusion criteria in the form of duplication, articles must be in English, experimental research, discussing ischemic stroke, and the experimental animals used are rats. The study used was only an experiment in mice because the application of the research in humans is still very limited. Additionally, homogeneous study participants may minimize the possibility of bias due to species differences.

Based on the selection, 37 articles were obtained that met the criteria. After further review, 19 articles were found suitable for this study because the complete article content and research reviewed curcumin’s neuroprotection effects, including its effect as antioxidant, anti-inflammatory, anti-apoptotic, and BBB protectant.

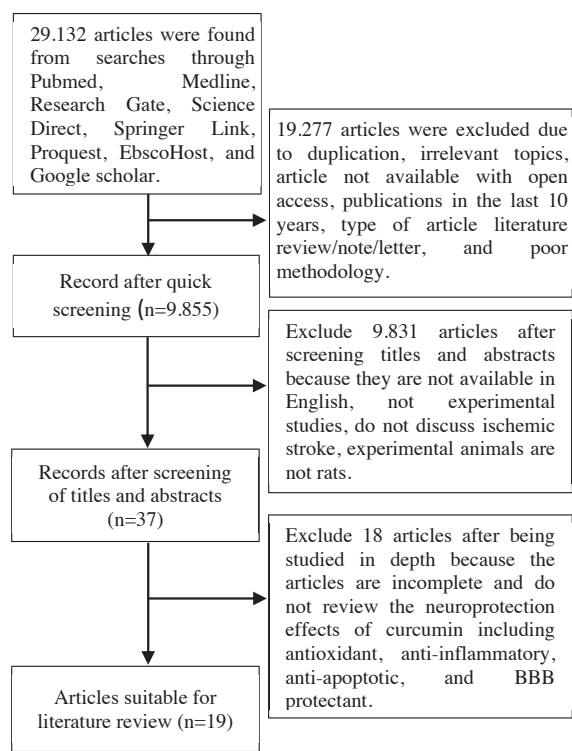


Figure 1. Flowchart of Article Selection

Figure 1. Flowchart of Article Selection

Table 1. Summary of Experimental Study of Curcumin in Mice

No	Study	Method	Dosage	Duration (week/s)	Result	Outcome	Reference
1	An experimental study using Female Sprague Dawley rats	Rats divided into five groups: normal group, untreated ischemic stroke (CIR) group, ischemic group with empty PLGA-PEG, FC (curcumin free) 5 mg/kg body weight, and NC (nano-encapsulated curcumin) 5 mg/kg body weight, containing 200 g of curcumin, administered orally	FC: 5 mg/kg body weight, NC: 5 mg/kg body weight, contains 200 g of curcumin		NC treatment group provided better neuroprotection by lowering ROS and preventing apoptosis than other groups (p<0.01) NC treatment group significantly decreased COX-2 and iNOS expression (p<0.05) NC treatment group significantly prevented the activation of caspase 3 and 9 NC treatment group significantly decreased lipid peroxidation (p<0.05) Nano-Curcumin (NC) is more effective in preventing cerebral ischemia than the Free-Curcumin (FC) form	Antioxidant Anti-apoptosis anti-inflammatory	(Mukherjee et al., 2019)
2	An experimental study using mice	Mice divided into three groups, namely the negative control group, the treatment group induced ischemic stroke using the middle cerebral artery occlusion (MCAO) and vehicle (VEH) methods, and the ischemic stroke group given curcumin (100, 200, 300, and 400 mg/Kg) through injection.	(100, 200, 300, and 400 mg/kg)		Curcumin group had a significant decrement in infarct volume compared to the VEH group (p<0.005) A dose of curcumin 300 mg/kg had the most significant effect on reducing infarct volume Curcumin-treated mice significantly inhibited increased expression of Bax, Caspase-3, and decreased expression of Bcl-2 (Anti-apoptotic)	Anti-apoptosis	(Xie et al., 2018)
3	An experimental study using Male SHR rats	SHR rats divided into control group (saline) and the curcumin group (100 mg/kg/day)	100 mg/kg body weight /day	Four weeks	Treatment group given curcumin significantly slowed the occurrence of ischemic stroke (p<0.05) Curcumin can significantly reduce the value of ROS in treatment group (p<0.05) Curcumin increases plasma nitrate/nitrite levels and plasma SOD activity	Antioxidant	(Lan et al., 2018)
4	An experimental study using Sprague Dawley rats	Rats divided into four treatment groups: vehicle + sham, curcumin (50 mg/kg) + sham, vehicle + MCAO, and curcumin (50 mg/kg) + MCAO. Each group consisted of 14 rats	50mg/kg body weight		Curcumin lowers the neurological deficit score	BBB Protectant	(Shah et al., 2016)

No	Study	Method	Dosage	Duration (week/s)	Result	Outcome	Reference
5	An experimental study using Male Wistar rats	Rats divided into four groups: control group (SO), ischemic group not given curcumin, treatment group (curcumin 25 mg/kg BW), and treatment group (curcumin 50 mg/kg BW) orally	25 mg/ body weight, 50 mg/ body weight	a week	COX-2 levels significantly decreased in the treatment group given curcumin 25 mg and 50 mg TNF- α levels in the treatment group given curcumin 50 mg were significantly lower than in the ischemic group not given curcumin	anti-inflammatory	(Alcântara et al., 2017)
6	An experimental study using male Sprague-Dawley rats	Mice divided into six groups: sham group, I/R group, Curcumin group, Curcumin + I/R group, I/R + siRNA group, and I/R + scrRNA group. Each group consisted of 8 mice. Curcumin is given by injection	300 mg/kg body weight	a week	Curcumin effectively reduced ROS and MDA levels in the treatment group ($p<0.05$) compared to the ischemic group	Antioxidant	(Jia et al., 2017)
7	An experimental study using Male Sprague-Dawley rats	Mice divided into five groups: untreated sham-operated, PBS-treated MCAO, nave Ex-treated MCAO, Free-cur treated MCAO, and Ex-cured (exosomes) treated MCAO	10 μ g/mL curcumin intravenous injection		Curcumin significantly decreased mitochondria-induced apoptosis Curcumin-treated mice inhibited Bax expression and significantly decreased Caspase-3, and caspase-9 Curcumin significantly reduces ROS levels Curcumin can significantly reduce tight junction protein	Anti-apoptosis Antioxidant BBB Protectant	(He et al., 2020)
8	An experimental study using Male SHR rats	Rats divided into four groups: sham group, ischemic/reperfusion group, solvent (dimethyl sulfoxide) control group, and curcumin treatment group	100 mg/kg	a week	The group given curcumin experienced a significant decrease in retinal cell apoptosis	Anti-apoptosis	(Wang et al., 2017)
9	An experimental study using female Wistar Hannover rats	Rats divided into five groups: (1) curcumin group orally administered before ischemia and intraperitoneally after ischemia, (2) curcumin group intraperitoneally after ischemia, (3) curcumin group orally before ischemia, (4) ischemic group, (5) sham group	300 mg/kg BW Oral and intraperitoneal	Three weeks	Superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) significantly higher in the curcumin I group ($p<0.05$) IL-6 and TNF- α significantly lower in the curcumin-treated group compared to the ischemic group ($p<0.05$) The treatment group given curcumin had a significantly lower apoptotic index compared to non curcumin group	Antioxidant anti-inflammatory Anti-apoptosis	(Altınay et al., 2017)

No	Study	Method	Dosage	Duration (week/s)	Result	Outcome	Reference
10	An experimental study using male Sprague-Dawley rats	Mice divided into three groups: CI (cerebral ischemic) given curcumin, vehicle group, and sham. Curcumin was started an hour after stroke and continued once a day for seven days	300 mg/kg by intraperitoneal injection	a week	• Curcumin can significantly reduce the post-stroke neurologic deficit	Reduce neurological deficit	(Liu et al., 2016)
11	An experimental study using male Sprague-Dawley (SD) rats	Rats divided into three groups: the sham group, the vehicle group, and the curcumin-treated group. Curcumin was administered intraperitoneally 30 minutes before surgery MCAO/R	300 mg/kg BW	1 week	Curcumin protects mice from impaired BBB permeability due to ischemic damage The group given curcumin before surgery significantly decreased MMP-9 and NF-κB levels	BBB protectant anti-inflammatory	(Wu et al., 2021)
12	An experimental study using male Wistar rats	Rats divided into four groups: the sham group was given a vehicle, the sham group was given curcumin, the ischemic stroke group was given a vehicle, and the ischemic stroke group was given curcumin	300 mg/kg BW by intraperitoneal injection		In the curcumin-treated MCAO group, infarct size decreased significantly The ischemic group treated with curcumin improved the neurological deficit score Curcumin significantly decreased ICAM 1 . expression Curcumin significantly decreased MMP-9 expression Curcumin significantly inhibits caspase-3 expression Curcumin reduces NF-κB expression	Reduce neurological deficit BBB Protectant Anti-apoptosis Anti-inflammatory	(Li et al., 2017)
13	An experimental study using adult male C57BL/6 mice	Mice divided into three groups: sham, ICH + vehicle, and ICH + curcumin	100 mg/kg		Curcumin significantly improves neurological scores	Reduce neurological deficit	(Liu et al., 2016)
14	An experimental study using male C57BL/6 mice	Mice assigned to 4 groups: sham, CI (cerebral ischemic), curcumin treatment 1, and curcumin treatment 2 + DKK1 (a blocker of Wnt receptor, 200 ng/d, icv)	Curcumin treatment 1 = 50, 100 mg/kg/day Curcumin treatment 2 = 100 mg/kg/day		Curcumin increases proteins expression involved in neurogenesis Significantly decreased T lymphocytes in the brain. Reduces T lymphocytes infiltration to the brain	Neurogenesis	(X. Yang et al., 2020)

No	Study	Method	Dosage	Duration (week/s)	Result	Outcome	Reference
15	An experimental study using male Sprague-Dawley rats	Experiments were carried out in 2 groups: MCAO (middle cerebral artery occlusion) and MCAO + CCM (curcumin)	50 mg/kg		Curcumin reduces TNF- α and IL-6 in brain Increase levels of Bcl-2 and reduce levels of Ap53 and Bax expression	Anti-apoptosis Anti-inflammatory	(Miao et al., 2016)
16	An experimental study using adult male Swiss albino mice	Mice divided into four groups with ten rats each: 1) sham, 2) control (stroke group), 3) vehicle, and 4) CUR treatment group.	100 mg/kg		IL-6, NF- κ B, MCP-1, and Bax levels lower in CUR group than in the control group An increase in BCL-2 in the CURgroup compared to the control group.	Anti-apoptosis Anti-inflammatory	(Hussein et al., 2020)
17	An experimental study using adult male C57BL/6J mice	Mice divided into three groups: sham, MCAO+vehicle, and MCAO+curcumin (n=8). All mice were used to measure infarct volume, neurobehavioral tests, white matter injuries, and protein expression	150 mg/kg BW	3 weeks	Curcumin inhibits the activation of the NF- κ B pathway Curcumin lowers the pyroptosis-related proteins, namely IL-1 β	Anti-inflammatory	(Ran et al., 2021)
18	An experimental study using male albino Wistar strain rats	Male albino rats divided into three groups with 6 in each group: 1) sham, 2) MCAO (Middle Cerebral Artery Occlusion), 3) MCAO + CUR	25mg/kg BW		The use of curcumin decreased IL-6 and TNF- α significantly. MMPs decreased significantly after CUR treatment. p53 and Bax proteins decreased significantly Bcl-2 increased on CUR treatment	Anti-apoptosis Anti-inflammatory	(Zhang et al., 2017)
19	An experimental study using adult male mice C57BL/6	Experimental animals divided into three groups: 1) sham, 2) stroke plus vehicle, 3) stroke plus curcumin groups. A total of 80 mice, 23 mice for infarct volume measurement. 42 mice for behavioural tests and immunohistochemical examination. 15 mice for measurement of mRNA or protein expression	150mg/kg immediately after ischemia and 24 hours later	1,5 weeks	Post-treatment curcumin reduced cerebral ischemic damage significantly within three days after dMCAO Curcumin treatment reduces the expression of pro-inflammatory cytokines, including TNF- α and IL-6	Anti-inflammatory	(Liu et al., 2017)

RESULTS AND DISCUSSION

From the results of the literature review, curcumin has a significant effect on ischemic stroke. Curcumin shows a number of roles that affect a brain with ischemic stroke, namely as an antioxidant, anti-apoptotic, anti-inflammatory, BBB protector, neurogenesis, and reducing neurological deficit.

Of the 19 articles reviewed, there were 5 articles which stated that curcumin has a high antioxidant content and can prevent significant nerve cell damage. The antioxidant properties of curcumin are characterized by a decrease in Reactive Oxygen Species (ROS) and lipid peroxidase, and an increase in superoxide dismutase (SOD) observed in this study. There were 10 articles that stated that the bioactive compound curcumin has significant anti-inflammatory properties. This anti-inflammatory property was inferred from the results of the research articles that showed a decrease in iNOS, COX-2, NF-B, TNF-, IL-1β, MMPs, an increase in IL-4, IL-10, and IGF-1. 9 articles showed positive results that curcumin has significant anti-apoptotic properties against brain cells as indicated by a decrease in the enzymes caspase 10, p53 and bax, caspase-9 and an increase in Bcl-2. A total of 3 articles showed the results that cur-

cumin was able to protect blood brain barrier (BBB) significantly by reducing degradation of tight junctions, production of MMP-9 and ICAM-1. In addition, 3 articles stated that curcumin can reduce neurological deficits and 1 article stated that curcumin has a positive effect on neurogenesis.

Ischemic stroke is a condition of blood flow decrement caused by blockage due to embolism or thrombus in the blood vessels of the brain. Blockage of cerebral blood vessels leads to oxygen and glucose deficiency which causes an imbalance of ion in the cells. This condition causes an increase in glutamate secretion, triggering toxic to neuron cells or commonly called excitotoxic which results in cell damage.

Curcumin is a bioactive compound that can prevent damage to neuron cells as a result of ischemic stroke. It has high antioxidant properties, anti-inflammatory properties, anti-apoptosis, and is able to protect the blood brain barrier (BBB). In addition, many studies showed that curcumin plays role in promoting neurogenesis and as neuroprotection useful for repairing brain cells in ischemic stroke patients (Li et al., 2016; Li et al., 2015; Rusek & Czuczwar, 2021; Ułamek-Kozioł et al., 2020).

Curcumin as an antioxidant

Ischemic stroke can trigger an increase in level of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) which increasing oxidative stress in brain cells, thereby triggering neurodegeneration (Daniel et al., 2018; Y. Wang et al., 2019). In addition, post-ischemic stroke involved glutamate excitotoxicity, causing calcium overload, resulting in mitochondrial function (Scholpa & Schnellmann, 2017). Mitochondrial dysfunction caused increased oxidative stress, increased catalase and superoxide dismutase (SOD)

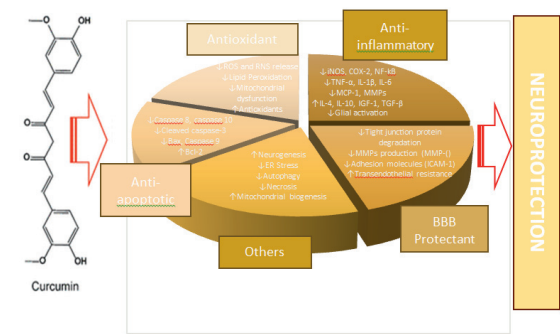


Figure 2. Curcumin Roles in Ischemic Stroke

enzymes, lipid peroxidation, DNA damage, and cell death (Awad, 2011; Subedi & Gaire, 2021).

Curcumin given after ischemic stroke was significantly able to prevent mitochondrial dysfunction by preventing glutamate excitotoxicity and preventing an increase in intracellular calcium. Curcumin was also able to reduce oxidative stress level by suppressing the number of ROS, RNS, and NO, thereby preventing neurodegeneration in brain nerve cells (Subedi & Gaire, 2021).

Curcumin as anti-inflammation response

Blockage of blood vessels in the brain which then developed into an ischemic stroke caused ion imbalance leading to excess glutamate in nerve cells (Godínez-Rubí et al., 2013). Excessive glutamate triggered an increase in intracellular calcium (Gaire et al., 2014). This condition caused increased iNOS production and pro-inflammatory cytokines such as TNF- α , IL-1, IL-6, and MMPs. This condition may exacerbate neuroglial cell activation, causing inflammation (Wang et al., 2019).

Curcumin is a bioactive compound with anti-inflammatory properties that is able to lessen inflammation mediators, such as proinflammatory cytokines, chemokines and adhesion molecules residing in brain. Curcumin can inhibit glial cell activation as well as the production of IL-1 β , IL-6, TNF- α , NF- κ B, MMPs (Li et al., 2015; Peng et al., 2021; Zhou et al., 2020). It can also significantly boost the production of anti-inflammatory cytokines namely IL-4, IL-10, and IGF-1. Therefore, it has a positive effect in suppressing the risk of brain inflammation due to ischemic stroke (Liu et al., 2017).

Curcumin as anti-apoptosis

Apoptosis is a common condition of cell death that can be caused as ischemic stroke (Uzdensky, 2019). High ROS exposure and excess calcium (Ca²⁺) cause increased oxidative stress and damage to the mitochondrial membrane so that Bax from the cytosol goes to the mitochondria (Sarmah et al., 2019; Sekerdag et al., 2018). High oxidative stress affected the production of pro-apoptotic proteins and inhibition of anti-apoptotic protein Bcl-2. In addition, high ROS exposure caused damage to mitochondria function and triggered

the activity of cytochrome c which released to cytosol and causes the formation of apoptosomes. Apoptosome formation caused procaspase-9 to be activated which triggered procaspase-3 to undergo activation. Activated caspase 3 can affect DNA fragmentation (Bavarsad et al., 2019). This is what then causes brain cell death.

Curcumin is a bioactive compound that can prevent cell death due to ischemic stroke. Pro-apoptotic proteins, namely Bax, Bak, and Caspase 3 and 9 were significantly inhibited by curcumin (Xie et al., 2018). In addition, curcumin also activated the Bcl-2 and reduced translocation of cytochrome c to cytosol to attenuate brain cell death activity (Li et al., 2017).

Curcumin protects Blood Brain Barrier (BBB)

Blood Brain Barrier (BBB) damage can cause brain cell damage after an ischemic stroke. BBB damage is characterized by tight junction proteins (ZO-1, claudin-5, occludin) damage located in cerebral blood vessels between endothelial cells. Tight junction proteins serve as gateways for immune cells and fluids (Jiang et al., 2018). Ischemic stroke followed by microglial cell activation leads to increased production of proinflammatory cytokines, thereby activating intercellular and vascular cell adhesion molecules (ICAM and VCAM) (Jiang et al., 2018). Adhesion molecules helped in the entry of immune cells into brain nerve cells (Yang et al., 2009). This process caused the degradation of the binding of tight junction proteins causing the BBB to become separated and edema to occur in brain cells.

Curcumin can provide protection to Blood Brain Barrier by increasing protein of tight junction. Curcumin also significantly decreased the enzyme matrix metalloproteinase (MMP) which was proteolytic towards the basement membrane (Dang et al., 2013; Wang et al., 2019), as well as VCAM and ICAM adhesion molecules, thereby preventing leukocyte infiltration in brain cells (Wicha et al., 2020). Decreased expression of VCAM and ICAM can also prevent inflammation of cerebral blood vessels thereby reducing the rate of brain injury (Funk et al., 2013). Thus, curcumin effectively has neuroprotective properties after ischemic stroke.

Curcumin improves neurogenesis

Administration of curcumin can significantly improve neurogenesis in brain hippocampal cells (Sun et al., 2020). Curcumin is neuroprotective and has a high antioxidant content. Increased hippocampal neurogenesis is evidenced by the ability of curcumin to initiate continuous proliferation of Neural Stem Cells (NSC) and form new neurons (Sun et al., 2020). The role of curcumin in enhancing neurogenesis consisted of various ways including activating the Wnt/ β -catenin signaling pathway. In addition, curcumin can also increase gene expression that play a role in cell proliferation (nesin, pax6, and reelin), neuron differentiation (Stat3, neuregulin, neurogenin, neuroD1, and neuroigin), and can activate extracellular signal regulated kinases and p38 kinases (Tiwari et al., 2014; X. Yang et al., 2020).

Encapsulated curcumin nanoparticles were more effective in the neurogenesis process compared to directly-consumed curcumin because curcumin has low bioavailability in brain cells (Tiwari et al., 2014). The administration of 30 mg/kg body weight curcumin showed positive results for the optimal neurogenesis process (Sun et al., 2020).

Curcumin reduces neurological deficits

Ischemic stroke is a condition of blockage of blood vessels in the brain that can cause neurological function disorders (Luo & Rubinsztein, 2013). Neurological deficits were caused by decreased neuronal function due to inflammation, high level of ROS, and apoptosis in brain nerve cells (Joshi & Johnson, 2012). Curcumin has significant anti-inflammatory, antiapoptotic, and antioxidant properties, thus it can reduce neuronal dysfunction in the brain which can prevent a more severe functional decline in the body.

Based on this review of 19 articles, the main finding is that curcumin has an absolute effect to post-ischemic stroke brain conditions. Curcumin is a bioactive compound with significant anti-inflammatory, antiapoptotic, and antioxidant properties. This results in agree to previous studies stating curcumin as a traditional medicine

has anti-inflammatory, anti-infective, antitumor, antibacterial, and antioxidant properties, and is believed to have a positive effect on ischemic stroke (Goozee et al., 2016; Hagl et al., 2015; Manca et al., 2015; Park et al., 2021; Rusek & Czuczwar, 2021). The anti-inflammatory, antioxidant and neuroprotective properties contained in curcumin help control ischemic stroke by preventing more severe brain impact (Ułamek-Kozioł et al., 2020). Limitation of this study are this article did not investigate the side effects of curcumin or the specific dose amount that can be beneficial in stroke circumstances. More research is needed to determine the appropriate dosage for ischemic stroke patients.

CONCLUSIONS

The bioactive compound curcumin has a positive effect in maintaining post-ischemic stroke conditions in rats. The drawback of this research is that it does not carry out a quantitative meta-analysis. In the future, it is hoped that clinical trial studies in humans can be carried out so that the effective dose and side effects of curcumin can be determined.

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ANALYSIS OF WATER CONTENT, FIBER, ANTHOCIANIN AND ORGANOLEPTIC ACCEPTANCE OF BUTTERFLY PEA JELLY POWDER (GATELA) AT VARIOUS DRYING TEMPERATURES OF SEAWEED

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ABSTRACT

Jelly is a kind of food that consumers typically of all ages like. Food additive ingredients can increase the nutritional content while providing an attractive color to the food. Butterfly pea flowers have the potential to be developed as a local natural food colorant while providing antioxidant effects. This research aims to determine the organoleptic profile, water content, fiber and anthocyanin content of butterfly pea flower (Gatele) gelatin powder at various seaweed drying temperatures, carried out in February-March 2023. The treatments in the research were seaweed drying temperatures which were divided into 3, namely temperature 50°C, 60°C and 70°C. The initial stage is the preparation of the ingredients, then continued with making butterfly pea flower (Gatele) agar powder, as well as organoleptic testing. The best formulation is followed by analysis of water, fiber and anthocyanin content. This research involved 30 students as panelists with the parameters tested, namely color, aroma, texture and taste. The results of the organoleptic test showed that the selected butterfly pea flower (Gatele) agar powder was the most preferred, namely with a drying temperature of 50°C in terms of aroma and texture. The results of the analysis on butterfly pea flower agar powder (Gatele) with a drying temperature of 50°C were a water content of 12.18%, a fiber content of 4.93% and anthocyanin content of 154.84 ppm. This butterfly pea flower gelatin powder (Gatele) can be accepted and is used as a local food-based preparation. In further research, we can increase the level of preference for the four indicators and the content of butterfly pea flower agar powder.

Keywords: *Jelly, butterfly pea flower, seaweed, drying temperature*

INTRODUCTION

Indonesia is famously known as an archipelago country that has a high variety of marine resources. This country is located right below the equator line, making the area exposed to the sun more throughout the year which is known to be beneficial for seaweed growth (Firdaus, 2019). A known species of seaweed called *Eucheuma cottonii* is one of the most popular seaweed that is sold in the market of Bengkulu. It is famously made for a special dessert *dawet*, which is a mix of coconut milk, ice cubes and palm sugar syrup (Sholiha, 2020). Moreover, *Eucheuma cottonii* can be used for jelly, which is an already famous dessert among the people, so the value of the seaweed can be more excelled (Juarsa, 2019).

The appeal of food can be evaluated from four aspects: color, taste, aroma and texture. An addition of food ingredients can raise its nutritional value while giving the food a better color (Gracelia

et al, 2022). Butterfly flea flowers can be used as a local natural coloring agent while also acting as an antioxidant (Handito et al, 2022). However, a lot of people overlook the benefit, and only see it as ordinary wild vine (Charolina et al, 2022).

Food processing method like the desiccation procedure determines the quality and quantity of the food products (Nurhayati et al, 2022). Using oven for the drying process can be an alternative, as it's easy to adjust the temperature, and unlike the conventional drying method, it's not depending on the weather (Orilda et al, 2021). The drying process can alter the food quality in sensory aspects of color, aroma, texture and taste (Asiah et al, 2021).

The temperature in the drying process affects the color of the seaweed (Orilda et al, 2021). Apart of the sensory aspects, the quality change also occurs in chemical aspects, whether it's an increase or decrease in its chemical activities (Asiah et al,

2021). The high temperature in the drying process can decrease the water content. Meanwhile, a high level of water content in the seaweed affects the texture or the gel strengths. Jelly powders with low-level of water content are less likely to be damaged than the high-level one (Yolanda et al, 2020).

Furthermore, the food water content determines its shelf life (Herliyana et al, 2022). Diminished water content has the potential to deactivate enzymes, impede chemical reactions, and suppress microbial growth, ultimately leading to a deterioration in food quality. According to SNI 2802:2015 standards, jelly powder is expected to adhere to a maximum water content of 22% to meet quality criteria. Consequently, a thorough examination of the water content was conducted at the drying temperature of seaweed in butterfly pea flower (*Gatela*) jelly powder, focusing on the treatment that garnered the highest preference.

Elevated drying temperatures pose a notable risk to the degradation or impairment of anthocyanin content within butterfly pea flowers. Substantial reduction in anthocyanin observed at drying temperatures surpassing 60°C, as documented by Martini *et al.* (2020). The diminution of anthocyanin content significantly contributes to an intensified and darkened coloration. Consequently, the inclusion of butterfly pea flower emerges as a pivotal determinant influencing the coloration of agar. The hue of the gelatin matrix assumes paramount importance as a primary factor shaping consumer appeal. The resultant color spectrum is contingent upon the amalgamation of seaweed flower powder with agar powder, considering diverse temperatures utilized in the drying process of distinct seaweed specimens.

The impact of drying temperature on seaweed has been elucidated in studies, demonstrating an augmentation in the fiber content of the material (Kusuma et al., 2019). In light of the foregoing discussion, the incorporation of butterfly pea flowers, serving as a local natural coloring agent, stands as a deliberate endeavor aimed at enhancing the nutritional profile of gelatin. This augmentation aligns with the preferences of consumers, thereby rendering it more palatable for consumption. The primary objective of this investigation was

to ascertain the optimal drying temperature for seaweed, employing a comprehensive evaluation conducted by panelists. This evaluation encompassed an organoleptic assessment, incorporating indicators such as color, taste, texture, and aroma. Furthermore, a quantitative analysis of water, fiber, and anthocyanin content was executed to provide a nuanced understanding of the compositional attributes of butterfly pea flower jelly powder (Gatela).

METHODS

The investigation was conducted in the months of February and March 2023, involving the production of items at the Food Technology Science Laboratory within the Nutrition Department at Bengkulu Health Polytechnic. Subsequent to the manufacturing phase, organoleptic evaluations were performed at the Organoleptic Assessment Laboratory affiliated with the Health Polytechnic under the Ministry of Health, Bengkulu. Following the organoleptic assessments, the quantitative analysis of water, fiber, and anthocyanin content was executed at the laboratory facilities of Bengkulu University.

The essential materials employed in this study included *Eucheuma cottonii* seaweed, procured from Pasar Minggu. Stringent criteria for selecting the seaweed necessitated specimens with unblemished thalli and a yellowish-white hue. Butterfly pea flowers, sourced from the environs of Lempuing and Berkas Beach, were meticulously chosen based on their vibrant blue petals, undamaged and fully bloomed characteristics. The instrumental apparatus employed throughout the product manufacturing process comprised a gas oven, a “TANITA 2 kg” scale, a precision 0.01 g MH-Series pocket scale, an oven pan, a basin, an 80-mesh sieve, and a ZT-300 FOMAC grinder.

The compositional analysis entailed the examination of water content (per AOAC, 2005, and corroborated by Orilda et al., 2021), crude fiber (as per AOAC, 1995, also substantiated by Orilda et al., 2021), and anthocyanin levels (determined differentially based on pH, following the method proposed by Purwaniati et al., 2020). The reagents employed in these analyses included 0.3 N acid sulfate (H₂SO₄), ethanol, distilled water, n-hexane,

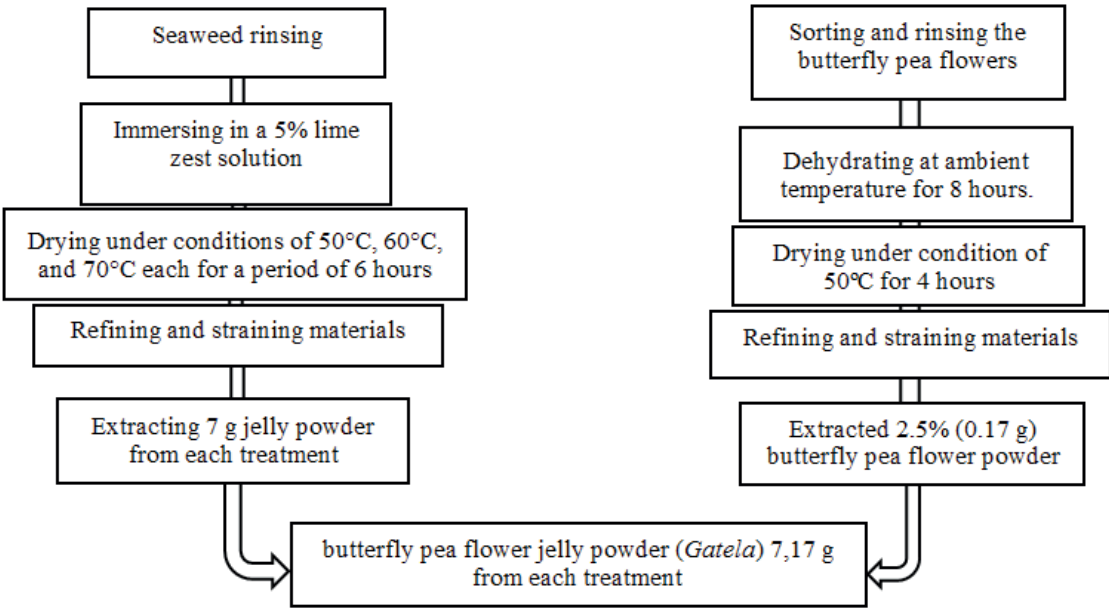


Chart 1. Process of Creating Butterfly Pea Flower Gelatin Powder

methanol, 15 N NaOH, HCl 1%, KCl pH 1.0, and sodium acetate pH 4.5. The requisite apparatus encompassed a cup, desiccator, reflux tube, beaker, filter paper, oven, furnace, and vial.

Ethical approval for this investigation was obtained from the Ethics Commission of Bengkulu Ministry of Health Polytechnic under Reference No. KEPK.BKL/139/04/2023. The research design employed is experimental, specifically characterized as experiment research, with the primary objective of evaluating a treatment pertaining to variations in seaweed drying temperatures—specifically, at 50°C, 60°C, and 70°C, as detailed in the work by Orilda et al. (2021). The overarching aim of this study is to discern the outcomes of analytical tests on water content, fiber content, and anthocyanin content within butterfly pea flower (*Gatela*) agar powder, the organoleptic qualities of which have been rigorously examined. The experimental treatments were systematically administered through the application of diverse seaweed drying temperatures, namely 50°C, 60°C, and 70°C.

The organoleptic assessment involved 30 semi-trained panelists, selected based on specific criteria encompassing satiety, willingness, absence of color blindness, overall health, and absence of illness. Evaluations transpired at the Food Laboratory

of Poltekkes Kemenkes Bengkulu. Each distinct jelly sample was presented in coded cups, with predetermined codes corresponding to the seaweed drying temperatures employed, namely 50°C (coded as 159), 60°C (coded as 274), and 70°C (coded as 315). Panelists independently assess the provided samples, responding to a questionnaire based on personal judgment devoid of external influences. Prior to tasting successive samples, panelists cleanse their palate with supplied water. Organoleptic evaluations encompass assessment of color, aroma, taste, and texture. The ordinal ranking sequence employed for organoleptic test evaluations of the four parameters is as follows: 1 = strongly dislike, 2 = dislike, 3 = somewhat like, 4 = like, and 5 = strongly like. Subsequently, the organoleptic test outcomes for the identified products are subjected to analysis, focusing on water, crude fiber, and anthocyanin content, within the laboratory facilities of Bengkulu University (UNIB).

The analytical scrutiny of data derived from organoleptic assessments was employed Kruskal-Wallis test. In the event that the initial analysis yields a significance level (p-value) below 0.05, further examination will be undertaken utilizing the Mann-Whitney test for more nuanced insights.

RESULTS AND DISCUSSION

Color

Upon scrutinizing the evaluations provided by the 30 panelists, it was discerned that 17 individuals exhibited a preference for the coloration of butterfly pea flower jelly (Gatela) subjected to a seaweed drying temperature of 70°C. The outcomes of the Kruskal-Wallis test, conducted to appraise the treatments, revealed no statistically significant impact on the organoleptic attributes related to the color of butterfly pea flower (Gatela) jelly (p-value = 0.143, $p > 0.05$). Consequently, the execution of the Mann-Whitney test was deemed unnecessary. The empirical findings indicate that, at a seaweed drying temperature of 70°C, the average assessment value from the panelists who expressed a preference for the color of butterfly pea flower jelly (Gatela) stands at 3.83.

The present investigation delves into the discernible impact of seaweed drying temperatures on the chromatic manifestation of butterfly pea flower jelly, known as “Gatela.” The alterations in coloration are inherently linked to the elevated drying temperatures, a phenomenon elucidated by the degradation of chlorophyll within the seaweed matrix, resulting in a concomitant diminution in luminosity (Widyastuti, et al., 2021). Furthermore, the infusion of butterfly pea flowers, replete with anthocyanin content, into the seaweed jelly substrate precipitates an additional transformative effect, ultimately inducing a discernible shift towards a purplish hue.

Noteworthy is the absence of statistically significant disparities in the coloration of the butterfly pea flower seaweed jelly powder (Gatela) across varied drying temperatures. Although perceptible distinctions in color intensity were

observed upon visual inspection—manifesting as a slightly deepened purple hue at 50°C, a characteristic purple hue at 60°C, and a marginally brighter purple hue at 70°C—these distinctions failed to attain statistical significance.

Aroma

Upon scrutiny of evaluations provided by 30 panelists, it was ascertained that 18 individuals expressed a favorable disposition toward the aroma of butterfly pea flower jelly (Gatela) when subjected to a seaweed drying temperature of 50°C. Employing the Kruskal-Wallis test to scrutinize treatment effects on the organoleptic attributes of the aroma, a calculated p-value of 0.397 ($p > 0.05$) was derived, signifying a lack of statistical significance. Consequently, the Mann-Whitney test, which would have further interrogated potential distinctions, was deemed unnecessary. The research findings elucidate that, at a seaweed drying temperature of 50°C, the average panelist score for aroma preference stood at 3.60, underscoring a noteworthy predilection for the olfactory characteristics of butterfly pea flower jelly (Gatela).

The findings of the investigation indicate a lack of discernible disparity in the organoleptic aroma preferences among the panelists, notwithstanding variations in seaweed drying temperature. *Eucheuma cottonii*, the seaweed under examination, is acknowledged for its intrinsic alkaline nature and characteristic piscine odor owing to the presence of ammonia. Intriguingly, alterations in seaweed drying temperatures did not yield concomitant changes in the aromatic profile exhibited by the butterfly pea flower agar (Gatela). This resilience in aroma consistency is attributed to the mitigating influence of a lime-infused

Table 1. Mean Organoleptic Evaluation of Butterfly Pea Flower Jelly Powder (Gatela) under Varied Drying Temperatures of Seaweed Substrates

No	Organoleptic Criteria	Temperature			p-value
		50°C (159)	60°C (274)	70°C (315)	
1	Color	3.47	3.73	3.83	0.143
2	Aroma	3.60	3.43	3.47	0.397
3	Texture	3.80	3.03	1.53	<0.001
4	Taste	3.30	3.60	2.73	<0.001

soaking process, as elucidated by Rosalita et al. (2018), the amalgamation of acidic compounds resulting from lime soaking serves to neutralize the inherently fishy aroma, effecting a diminution or even complete elimination thereof.

Texture

Upon analysis of evaluations provided by 30 panelists, a noteworthy observation emerged, indicating that 18 individuals expressed a favorable inclination towards the gelatinous texture of butterfly pea flowers (Gatela) when subjected to a seaweed drying temperature of 50°C. Employing the Kruskal-Wallis test to scrutinize treatment effects on the organoleptic attributes of the texture yielded a strikingly significant outcome, as evidenced by a p-value of <0.001 ($p < 0.05$). Consequently, a subsequent Mann-Whitney test was executed to delve deeper into the nuances of observed distinctions. The research outcomes elucidate that, at a seaweed drying temperature of 50°C, the average panelist rating for texture preference stood at 3.80, affirming a discernible proclivity for the gelatinous quality of butterfly pea flower jelly (Gatela).

The outcomes of the investigation revealed a discernible influence of diverse seaweed drying temperatures on texture characteristics. Comparative analysis of textures arising from three distinct seaweed drying temperatures applied to Telang flower jelly (Gatela) unveiled a statistically significant impact on agar texture ($p < 0.001$). Intriguingly, higher drying temperatures yielded a gelatinous texture characterized by reduced density and chewiness. This observed phenomenon is attributed to the consequential degradation of carrageenan polymer chains within the seaweed matrix at elevated drying temperatures, leading to a decline in viscosity and subsequently impacting gel formation. The attenuated durability or strength of carrageenan, stemming from its diminished viscosity, is countered by higher carrageenan content, imparting greater repulsion and fortitude to the resulting gel, as expounded by Pratiwi et al. (2022). Complementing these findings, Uju et al. (2018) reported the highest viscosity value at a seaweed drying temperature of 50°C.

Upon the continuation of the Mann-Whitney test for butterfly pea flower seaweed jelly powder

(Gatela) at varying seaweed drying temperatures, specifically 50°C (159) versus 60°C (274), a discernible difference in texture was uncovered, evidenced by a statistically significant p-value of <0.001 ($p < 0.05$). Similarly, a distinct textural contrast emerged between butterfly pea flower seaweed jelly powder (Gatela) subjected to seaweed drying temperatures of 50°C (159) and 70°C (315), substantiated by a p-value of <0.001 ($p < 0.05$). Further, the examination of textures in butterfly pea flower seaweed jelly powder (Gatela) under drying temperatures of 60°C (274) versus 70°C (315) elucidated a significant difference, as indicated by a p-value of <0.001 ($p < 0.05$). This observed textural variability is intricately linked to carrageenan, a polysaccharide inherent to seaweed, renowned for its utility as a thickening and gelling agent. Carrageenan's distinctive capacity to bind and retain water imparts a robust texture to food products, as underscored by Yudiastuti et al. (2023).

Taste

Following the evaluation conducted with a sample of 30 panelists, it was revealed that 18 individuals exhibited a preference for the flavor profile of butterfly pea flower jelly (Gatela) when subject to a seaweed drying temperature of 60°C. The application of the Kruskal-Wallis test yielded a statistically significant impact on the organoleptic attributes of taste in butterfly pea flower (Gatela) jelly, with a calculated p-value of <0.001 ($p < 0.05$). Subsequent to this observation, the Mann-Whitney test was executed to further elucidate the nature of the identified distinctions. The research findings underscore that, at a seaweed drying temperature of 60°C, the average rating assigned by panelists who favored the taste was 3.60, indicating a discernible inclination toward the flavor profile of butterfly pea flower jelly (Gatela).

Elevated drying temperatures exert a discernible influence on the firmness of the resulting gel, a phenomenon attributable to the presence of carrageenan within the seaweed matrix. Carrageenan, renowned for its water-binding capacity (hydrocolloids), plays a pivotal role in the gel formation process by effectively binding water-soluble sugars. This intrinsic property of carrageenan is expounded upon in the study by

Stevani et al. (2019), elucidating its capability to bind and retain water. Aligning with the findings of Fajarini et al. (2018), it is established that increased carrageenan content contributes to a discernible sweetness in black grape skin jelly candy (*Vitis vinifera*).

As elucidated in the exposition by Vania (2017), the augmentation of double helix polymer chains is notably pronounced in response to elevated concentrations of carrageenan. This heightened concentration imparts increased strength and robustness to the polymer structure. The substantial binding of water molecules, facilitated by carrageenan, further contributes to the fortification of the gel matrix, rendering it firmer and more resilient. Noteworthy, however, is the tasteless nature of carrageenan, as expounded upon in the literature, indicating its lack of influence on taste parameters.

Upon the continuation of the Mann-Whitney test for butterfly pea flower jelly powder (Gatela) at distinct seaweed drying temperatures—specifically, 50°C (159) versus 60°C (274)—discernible taste disparities were unveiled. The calculated p-value of 0.048 ($p < 0.05$) attests to the statistical significance of the identified taste differences at these temperatures. Similarly, the exploration of taste nuances in butterfly pea flower jelly powder (Gatela) subjected to seaweed drying temperatures of 50°C (159) versus 70°C (315) revealed significant taste distinctions, as indicated by a p-value of 0.004 ($p < 0.05$). Furthermore, the assessment of taste variances in butterfly pea flower jelly powder (Gatela) exposed to seaweed drying temperatures of 60°C (274) versus 70°C (315) confirmed a pronounced taste disparity, underscored by a p-value of 0.000 ($p < 0.05$).

Water content

The analysis of water content in butterfly pea flower jelly powder (Gatela) at a drying temperature of 50°C revealed a content of 12.18%. In alignment with the findings of Orilda (2021), there was an observed reduction in water content in dried *Eucheuma cottonii* seaweed across a range of drying temperatures (50°C - 70°C), registering at 15.87% - 10.69%. A comparative examination with the water content of butterfly pea flower herbal tea, dried at 50°C for 4 hours, disclosed a

content of 10.18% (Martini et al., 2020). Notably, despite the incorporation of butterfly pea flower, the water content in butterfly pea flower jelly powder (Gatela) adheres to established quality standards for water content in flour gelatin, as stipulated by SNI 2802:2015, which prescribes a maximum threshold of 22%.

The desiccation procedure applied to a material inherently leads to a reduction in water content. This reduction holds paramount significance, as higher water content in food renders it more susceptible to deleterious consequences such as microbial growth, biological activity, and the infiltration of detrimental bacteria, thereby exacerbating the risk of spoilage or deterioration (Herliyana et al., 2022). The inverse correlation between water content and the longevity of food underscores the pivotal role of the drying process in fortifying the stability and shelf-life of consumable products.

Crude Fiber Content

The analysis of crude fiber content in butterfly pea flower jelly powder (Gatela) subjected to a seaweed drying temperature of 50°C revealed a content of 4.95%, demonstrating a notable increase compared to the crude fiber content in fresh *Eucheuma cottonii* seaweed, which ranged from 2.25% to 2.82% (Safia et al., 2020). This finding aligns closely with the results of research by Khotijah et al. (2020), indicating a comparable crude fiber content of 3% in fresh *Eucheuma cottonii* seaweed. Additionally, insights from Kusuma et al. (2019) underscore the correlation between drying temperature and crude fiber content, elucidating a progressive increase in crude fiber levels with escalating drying temperatures.

The evaluation of crude fiber content in seaweed subjected to drying temperatures of 50°C, 60°C, and 70°C for a duration of 6 hours yielded values of 1.15%, 1.25%, and 1.4%, respectively (Orilda et al., 2021). This indicates a considerable deviation of 49.5% from the baseline research result of 1.15% (Orilda et al., 2021). Beyond the impact of drying temperature, the incorporation of crude fiber content from butterfly pea flowers introduces an additional dimension. According to research by Handito et al. (2022), fresh butterfly pea flowers boast a crude fiber

content of 5.5%. Consequently, the calculated crude fiber ratio is 3.8% higher than the findings of Orilda et al. (2021) at an equivalent seaweed drying temperature of 50°C.

The elevated crude fiber content within this jelly formulation holds promise in mitigating obesity and heart disease. The presence of fiber accelerates the expulsion of food waste through the digestive tract, contributing to overall digestive health. Furthermore, fiber exerts regulatory effects on blood glucose and blood cholesterol levels, thereby playing a pivotal role in metabolic control. Additionally, the inclusion of fiber has been associated with a reduction in the risk of cancer, as highlighted by Damayanti et al. (2020).

Anthocyanin Level

The analysis of anthocyanin content in butterfly pea flower jelly powder (Gatela) at a seaweed drying temperature of 50°C revealed a concentration of 154.84 ppm or 15.484 mg/100g. These findings, however, indicate a lower anthocyanin level compared to fresh butterfly pea flowers, which boasts a concentration of approximately 22.74 mg/100g (Kiranawati et al., 2022). This discrepancy is primarily attributed to the preservation of anthocyanins in fresh butterfly pea flowers, as they have not undergone processes such as drying or heating that may compromise or degrade these compounds, as elucidated by Purwaniati et al. (2020).

In contrast to the findings presented by Martini et al. (2020), the crude anthocyanin content in butterfly pea flower tea powder subjected to a drying temperature of 50°C for 4 hours reached 249.7 mg/100g. This discrepancy becomes more pronounced when considering that the study incorporated only 2.5% (0.17g) of butterfly pea flower tea powder, while Martini et al. utilized 1g. The observed lower anthocyanin content in butterfly pea flower (Gatela) agar powder at a drying temperature of 50°C is attributed to the natural degradation of anthocyanins during the storage process.

Elevated storage temperatures have been identified as a catalyst for anthocyanin degradation, leading to a consequential reduction in anthocyanin concentration. This phenomenon is underscored by the research findings of Permatasari (2021),

wherein buni fruit coloring powder stored at 55°C exhibited a more substantial decline in anthocyanin levels compared to storage at temperatures of 28°C and 35°C. While the current study did not explicitly manipulate storage temperature as a variable, the butterfly pea flower (Gatela) agar powder under investigation was stored at room temperature. It is noteworthy that room temperature can induce anthocyanin damage through oxidation, resulting in a subsequent decline in anthocyanin content, as elucidated by Nalawati et al. (2022).

Additives incorporating anthocyanins serve a dual purpose as natural food coloring agents, imparting vibrant colors to a spectrum of food and beverage products. Beyond their aesthetic contribution, anthocyanins play a crucial role in averting degenerative diseases. This is exemplified in their ability to combat cardiovascular diseases by mitigating and inhibiting cholesterol levels in the bloodstream, stemming from the oxidation of low-density lipoproteins (LDL), as evidenced by Priska et al. (2018).

CONCLUSION

The optimal choice for butterfly pea flower (Gatela) jelly powder is discerned at a seaweed drying temperature of 50°C. This preference aligns with the SNI standards, as the analysis indicates a water content of 12.18%, falling within the specified range for jelly flour. Further scrutiny reveals a crude fiber content of 4.95% and an anthocyanin content of 154.84 ppm.

To enhance product development, it is advisable to delve deeper into preferences, explore additional macro and micro-nutrient compositions, and meticulously examine the impact of varying temperatures and storage durations. A comprehensive comparative analysis between treatments and control variables will shed light on the nuanced intricacies influencing the overall quality of butterfly pea flower (Gatela) jelly, facilitating a more nuanced and informed approach to formulation.

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SUGAR CONTENT, CRUDE FIBER CONTENT, ANTIOXIDANT ACTIVITY, AND SENSORY CHARACTERISTICS OF SORGHUM (SORGHUM BICOLOR (L.) MOENCH) SNACK BAR WITH ADDITION OF KLUTUK BANANA (MUSA BALBISIANA COLLA) FLOUR

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ABSTRACT

Sorghum and klutuk banana can be utilized as functional foods that are high in fiber and antioxidant and suitable for diabetic people. This study examined the effects of klutuk banana flour on the sugar content, crude fiber content, antioxidant activity, and sensory characteristics of sorghum snack bars. This study is true experimental, employing a completely randomized design (CRD) with 4 formulations: the ratio of sorghum and banana flour klutuk PO (100:0), P1 (90:10%), P2 (80:20%), and P3 (70:30%), and 6 repetitions. Sugar content, crude fiber, and antioxidant activity were observed and analyzed using the one-way ANOVA (Analysis of Variance) test, followed by Duncan test. The sensory properties were evaluated using Friedman test. 1.94–2.86% sugar, crude fiber content is 46.04–70.89%, and antioxidant activity is 9.06–15.0%. The results revealed a significant relationship ($p < 0.05$) between the addition of klutuk banana flour to the sorghum snack bar sugar level, crude fiber content, and antioxidant activity. Texture, color, flavor, and scent were not significantly affected by the addition of klutuk banana flour to sorghum snack bars. The optimal formulation of a sorghum snack bar with the inclusion of klutuk banana flour was discovered to be P3 based on the test sugar content, crude fiber content, antioxidant activity, and sensory characteristics. The nutritional value of the P3 formulation have met USDA (2018) standards for the Nutri-Grain Fruit and Nut Bar, but the sugar content have not meet the SNI 01-3702-1995 (Indonesian National Standards) quality requirements for diabetes diet biscuit.

Keywords: antioxidant activity, diabetes mellitus, sugar, sensory characteristics, crude fiber, snack bar, klutuk banana flour

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by blood sugar levels above normal, and results in damage to insulin secretion, insulin action, or both (Akram, 2015). According to Basic Health Research (Riskesdas) data, the prevalence of diabetes mellitus in Indonesia in 2018 was 1.5% (Ministry of Health, 2018). The prevalence is estimated to increase to 578 million in 2030 and 700 million in 2045 (Ministry of Health, 2020).

Individuals with diabetes mellitus (DM) necessitate the incorporation of antioxidant sources into their dietary regimen to impede oxidative damage within the body (Tritisari et al., 2017). Additionally, meticulous regulation and monitoring of carbohydrate intake, both in terms of quantity and type, play a pivotal role in managing blood glucose level effectively (Surayya et al., 2020).

Guidance for individual with diabetes includes the adoption of a health-conscious diet, with a specific emphasis on augmenting fiber consumption as a means of blood glucose control (Azrimaidaliza, 2011). It is recommended that individual with diabetes aim for a daily fiber intake within the range of 20-35 gram (Indonesia Endocrinology Association, 2019).

Sorghum exhibits notable nutritional characteristics, boasting a fiber content of 6.7 gram per 100 gram (USDA, 2018). The seeds of sorghum contribute 7.34 gram of glucose per 100 gram (Diyah et al., 2018). Adhering to the regulatory framework outlined in Head of BPOM Regulation Number 13 of 2016 concerning Supervision of Claims on Processed Food Labels and Advertisements, a product can be designated as high in fiber if it attains a fiber content of 6 g/100 g or 6%. Moreover, sorghum emerges as

a commendable source of antioxidants, with an appreciable content of 40.46% (Wu et al., 2013). In the context of dietary consideration for individual with diabetes mellitus (DM), it is imperative not only to incorporate antioxidant-rich foods but also to introduce supplementary ingredients rich in fiber. Klutuk banana (*Musa balbisiana* colla) flour emerges as a viable option for enhancing fiber intake among individuals with DM. Traditionally utilized primarily for its leaves as wrappers due to their tear-resistant nature (Hastuti, 2021), Klutuk bananas exhibit superior nutritional attributes compared to other banana varieties. With a substantial crude fiber content of 6.9% (Endra, 2006), and a sugar content of 11.79% (Kehek, 2017), Klutuk banana provides a robust foundation for augmenting the dietary fiber required by individual with DM. Additionally, the antioxidant activity across various banana varieties ranges from 44.4 to 51.6 µg/mL (Siji & Nandini, 2017). This underscores the multifaceted nutritional benefit of incorporating Klutuk banana flour into the dietary regimen of individual managing diabetes mellitus.

Sorghum and Klutuk banana present promising prospect as constituent of functional food, particularly in the formulation of snack bars, offering an alternative dietary option replete with abundant fiber and antioxidant. Snack bars, characterized by their high fiber and low glucose composition, emerge as an especially suitable consumable for individuals, notably those managing diabetes mellitus (DM) (Zaddana et al., 2021). This study aims to advance the development of a functional snack in the form of a sorghum-based snack bar, enriched with the inclusion of Klutuk banana flour. The investigation further seeks to evaluate key parameters such as sugar content, fiber composition, antioxidant activity, and sensory characteristics of the developed snack bars, thereby contributing to a comprehensive understanding of their nutritional profile and palatability.

METHODS

This research adheres to the principles of true experimental design, employing the Completely Randomized Design (CRD) methodology

encompassing four distinct formulations denoted as P0 (100:0%), P1 (90:10%), P2 (80:20%), and P3 (70:30%). The study incorporates six replications for each formulation, determined through application of Federer's formula. The control formulation, designated as P0, serves as the reference standard against which experimental formulations are compared. The experimental procedures transpired in June 2022 within the confines of the Food Processing Laboratory at Muhammadiyah University Semarang, where the synthesis of klutuk banana flour and sorghum snack bars, featuring the incorporation of klutuk banana flour, took place. Concurrently, assessment of sugar content, fiber composition, and antioxidant activity were conducted at the Nutrient Analysis Laboratory of Muhammadiyah University Semarang. To further elucidate the sensory attributes of the sorghum snack bars augmented with klutuk banana flour, comprehensive evaluation were carried out at the Organoleptic Laboratory, Muhammadiyah University, Semarang. A discerning panel of 20 semi-trained individuals, specifically selected from students enrolled in the Undergraduate Nutrition and Food Technology program at Muhammadiyah University, participated in the sensory analysis, contributing valuable insights into the palatability and organoleptic dimension of the developed snack bars.

Materials and Tools

The raw materials utilized in this study were meticulously selected, comprising brown sorghum sourced from Batang Regency and klutuk bananas procured from Sembojo Village, Tulis District, Batang Regency, Central Java. The formulation included precise quantities of 5 gram of corn sugar, 15 gram of margarine, 20 gram of chicken egg yolk, 20 gram of skim milk flour, and 15 ml of water, as outlined by Zaddana et al. (2021). For chemical analyses, a comprehensive array of materials was employed, encompassing distilled water, luff-schrool solution, H₂SO₄ 6N solution, KI 30%, Na₂S₂O₃ 0.1 N, 1% starch, H₂SO₄ 0.325 N solution, NaOH 1.25 N solution, K₂SO₄ 10% solution, 95% ethanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution and methanol solution. These substances were instrumental in conducting various chemical tests, allowing for

a thorough examination of the nutritional and antioxidant properties of the developed snack bars.

The preparation and processing of ingredients used cabinet dryer, knife, cutting board, basin, baking sheet, disk mill, scale, stove, spoon, spatula, oven, and conventional popper. These tools ensured the efficient and standardized execution of the snack bar formulation and processing protocols. The chemical analyses were conducted using scale, porcelain cup, measuring flask, closed Erlemeyer, volume pipette, burette, measuring cup, filler, stamper mortar, Erlenmeyer, return cooler, stative, hot plate, beaker, dropper pipette, funnel, filter paper, desiccator, oven, vortex, test tube, tube rack, aluminum foil, vial, and spectrophotometer. Furthermore, the sensory evaluation component of the study employed tools such as writing instruments and hedonic test forms. The comprehensive array of tools employed in this study underscores the scientific rigor and methodological thoroughness applied to both the chemical and sensory evaluations.

Phase of Making Klutuk Banana Flour

The initial phase of Klutuk banana flour production entails the meticulous peeling of Klutuk banana skin, followed by the precision slicing of banana into thin section measuring 30 x 30 mm. Subsequently, the sliced banana undergo a water immersion process for a duration of 30 minutes. The rehydrated Klutuk bananas are then systematically arranged within a baking dish and subjected to a controlled drying process at 50°C in an oven, spanning a duration of 24 hours. Following the dehydration phase, the banana are finely ground to achieve the desired particle size using a disk mill equipped with an 80 mesh sieve, in accordance with the methodology outlined by Musita (2014). This systematic procedure ensures the production of Klutuk banana flour with optimal physical and compositional attributes for subsequent incorporation into the development of nutritionally fortified snack bars.

Rendered Results = $\frac{\text{final weight}}{\text{initial weight}}$ x 100%

Rendered Results = $\frac{945 \text{ gram}}{4.875 \text{ gram}}$ x 100%
= 19,38%

The yield of Klutuk banana flour was 19.38%.

Determining the Formula for Sorghum Snack Bars with the Addition of Klutuk Banana Flour

Table 1. Sorghum snack bar formulation with the addition of klutuk banana flour

Formulation	Brown Sorghum (%)	Klutuk Banana Flour (%)
P0	100*	0*
P1	90	10
P2	80	20
P3	70	30

Source : (Zaddana et al., 2021)

* (Rufaizah, 2011)

Phase of Making Sorghum Snack Bars with the Addition of Klutuk Banana Flour

The preliminary phase involves the preparation of sorghum pop, initiated by washing and subsequently utilizing a conventional popper as delineated by Linda and Panunggal (2015). Following this, the snack bar formulation commences with the amalgamation of wet ingredients, namely margarine, water, and chicken egg yolk. The amalgamated wet ingredients are then judiciously introduced to the dry components, encompassing sorghum pop, klutuk banana flour, skim milk flour, and corn sugar. The ensuing mixture undergoes a meticulous kneading process until achieving a homogenously smooth consistency, adhering to the methodology articulated by Zaddana et al. (2021). Subsequently, the resulting snack bar dough is expertly shaped into a rectangular form within a designated baking pan, in accordance with the procedural guidelines elucidated by Rufaizah (2011). The final transformative step involves the controlled baking of the molded dough in an oven set at 130°C, an operation spanning 30 minutes, aligning with the methodology stipulated by Fajri et al. (2020). This systematic and methodically detailed approach ensures the production of nutritionally fortified

sorghum and klutuk banana snack bars with precise physical and compositional attributes.

Chemical Properties Analysis

1. Sugar Level Test (Faizati et al., 2018)

A representative aliquot of 5 gram sample was quantitatively transferred into a volumetric flask, and distilled water was meticulously added to achieve the calibrated volume. Subsequently, a precisely measured 5 mL aliquot of the resultant sample solution was withdrawn and combined with 10 mL of Luff-Schrool solution. The amalgamated solution underwent a controlled reflux process for a duration of 10 minutes. Upon completion of refluxing, the solution was allowed to cool, and a subsequent addition of 10 mL of 6N H₂SO₄ solution and 10 mL of 30% KI was carried out. The ensuing solution was subjected to a meticulous titration protocol utilizing 0.1 N Na₂S₂O₃ until the manifestation of a distinct straw-yellow color. Once the solution attained the predetermined straw-yellow hue, 1 mL of 1% starch solution was judiciously introduced into the system. Following this addition, titration resumed until the color transitioned to a milky-white appearance. This methodological approach ensures the precise determination of chemical constituents, employing titration techniques to establish quantitative endpoints indicative of the desired chemical reactions.

$$a = \frac{(V \text{ blanko} - V \text{ sample}) \times \text{real N Na}_2\text{S}_2\text{O}_3}{\text{N Na}_2\text{S}_2\text{O}_3 \text{ 0,1 N}}$$

$$\% \text{ sugar} = \frac{b \times \text{FP}}{W \text{ (g)} \times 1000} \times 100\%$$

Note:

Value a = value b obtained using the help of the Luff Schrool table.

2. Fiber Level Test (AOAC, 2005)

A precisely measured quantity of 1 gram of the sample was meticulously introduced into an Erlenmeyer flask, followed by the addition of 50 mL of 0.325 N H₂SO₄. Subsequently, the mixture underwent a controlled reflux process for a duration of 30 minutes. To this resultant mixture, 50 mL of 1.25 N NaOH solution was incrementally added, and the reflux process was continued for

an additional 30 minutes. The ensuing reaction mixture was subjected to filtration using Whatman filter paper, ensuring the separation of the solid residue from the liquid phase. The residual material on the Whatman filter paper was subjected to a sequential washing regimen. Initially, the residue was washed with 25 mL of distilled water, followed by an additional wash with 25 mL of 10% K₂SO₄ solution. Subsequent rinses included a wash with 25 mL of distilled water, and a final wash with 20 mL of 95% ethanol. The residue, now purified, was then subjected to drying in an oven maintained at 105°C for a stipulated period of 2 hours. Following the drying process, the sample was placed within a desiccator for 15 minutes to equilibrate moisture level, after which it was meticulously weighed. This systematic series of procedures ensures the preparation, purification, and quantitative analysis of the sample in a controlled chemical environment.

$$\text{Fiber Content (\%)} = \frac{\text{final weight} - \text{initial weight (gram)}}{\text{sample weight (gram)}} \times 100\%$$

3. Antioxidant Activity Assessment (Chandra, 2010)

In the pursuit of assessing antioxidant activity, the 0.1 mM DDPH method was employed. A precisely measured 0.5 gram aliquot of the specimen underwent dissolution in a meticulously dispensed 10 ml volume of methanol within a test tube, diligently shielded with aluminum foil. Subsequently, the sample was subjected to vigorous vortexing for a duration of 1 minute, followed by an extraction period of 3 hours. For the subsequent analytical step, a precisely measured 0.2 mL aliquot of the resulting sample solution was introduced into a test tube, enveloped in aluminum foil. To this, 3.9 mL of a meticulously prepared 0.1 mM DPPH solution was added, and the amalgam was subjected to thorough vortexing. This amalgamation was then allowed to stand for a duration of 30 minutes. Following this incubation period, the absorbance of the sample was quantified at a wavelength of 517 nm, employing the precision of a UV-Vis spectrophotometer. Concurrently, a blank sample was meticulously prepared using 0.2 mL of methanol and 3.9 mL of the 0.1 mM DPPH solution, following a parallel

methodology. This methodological rigor ensured a comprehensive evaluation of antioxidant activity, underscoring the meticulousness inherent in the experimental design and execution.

(% inhibition) = $\frac{\text{blank absorption} - \text{sample absorption}}{\text{blank absorption}} \times 100\%$ |

Data Analysis

In the domain of nutritional assessment, an examination of data pertaining to sugar content, crude fiber, and antioxidant activity commenced with the utilization of the Shapiro-Wilk statistical test to evaluate normality. This meticulous analysis revealed that the data pertaining to sugar content, crude fiber, and antioxidant activity exhibited normal distributions. Subsequently, a one-way Analysis of Variance (ANOVA) test was employed, followed by a post hoc Duncan test for further discernment of potential variances among the means. This approach was taken to explore and elucidate the nuanced variation within the normal dataset. Conversely, the sensory characteristics data yielded non-normally distributed outcomes. In response to this departure from normality, the Friedman test, a non-parametric alternative suitable for assessing repeated measures, was judiciously employed. This test was selected to discern and expound upon any discernible differences in the sensory characteristics that may not conform to a normal distribution. Through this multifaceted statistical approach, a thorough and nuanced understanding of the nutritional and sensory aspects under investigation was achieved, embodying the methodological precision inherent in medical academic research.

RESULTS AND DISCUSSION

Snack bars emerge as a nutritionally sugar content in brown sorghum establishes it as a

prudent dietary choice for individuals contending with degenerative conditions like diabetes.

A comparative analysis with Klutuk banana reveals a decline in sugar content even in its flour form. Klutuk banana fruit registers a sugar content of 11.78%, whereas Klutuk banana flour records a substantially reduced to 2.78% (Kehek, 2017). The decreased sugar content in the banana flour is attributed to the utilization of raw klutuk bananas and the subsequent drying process at a temperature of 50°C. The elevated temperature induces the expansion of starch granules, leading to the dissolution of the low amylose fraction and uniform breakage of starch granules, potentially causing carbohydrate modification, as explicated by Kurniawan et al. (2015).

As per USDA data from 2018, sorghum demonstrates a fiber content of 6.7 gram per 100 gram, while the current study records a slightly elevated value of 8.39%. In the case of Klutuk banana flour, the crude fiber content exceeds that of its fruit counterpart by 44.70%. Endra (2006) reports a crude fiber content of 6.90% in Klutuk bananas. This divergence arises from the drying process employed in Klutuk banana flour production, as elucidated by Simanjuntak et al. (2013), wherein elevated drying temperatures lead to reduced water content, subsequently causing an augmentation in crude fiber content concomitant with an increase in carbohydrate content. It is imperative to note that fiber, a complex carbohydrate inherent in food, plays a crucial nutritional role (Simanjuntak et al., 2016).

The antioxidant activity of sorghum, as reported by Wu et al. (2013), stands at 40.46%, diverging significantly from the findings of Linda and Panunggal (2015) where brown sorghum exhibits an antioxidant activity level of 95.38%. Discrepancies in antioxidant activity level can be attributed to geographical and climatic variation in the locales of ingredient sourcing, influencing the composition of bioactive compounds,

Table 1. Test Results for Sugar Content, Crude Fiber Content, and Antioxidant Activity in Brown Sorghum and Klutuk Banana Flour

Nutriment	Sugar (%)	Crude Fiber (%)	Antioxidant Activity (%)
	Mean ± standard deviation	Mean ± standard deviation	Mean ± standard deviation
Brown Sorghum	1.33 ± 0.22	8.39 ± 0.27	46.5 ± 0.74
Klutuk banana flour	2.78 ± 0.28	44.70 ± 1.54	27.21 ± 1.69

Table 2. Sugar Content, Fiber, and Antioxidant Activity of Sorghum Snack Bars with the Addition of Klutuk Banana Flour

Formulation	Sugar Content (%) Mean ± standard deviation	Crude Fiber Content (%) Mean ± standard deviation	Antioxidant Activity (%) Mean ± standard deviation
P0	1.57±0.48 ^a	28.46±1.11 ^a	5.00±2.03 ^a
P1	1.94±0.51 ^{ab}	46.04±3.93 ^b	9.06±2.74 ^b
P2	2.19±0.12 ^b	52.49±1.47 ^c	12.07±2.51 ^{bc}
P3	2.86±0.58 ^c	70.89±4.91 ^d	15.05±2.86 ^c

Note: Formula P0 = (100%:0%), P1 = (90%:10%), P2 = (80%:20%), P3 = (70%:30%)

Values with different lowercase superscripts in the same column indicate significant differences (p<0.05)

particularly flavonoid, phenolic compound, and tannin (Supriatna et al., 2019). The antioxidant activity of Klutuk banana flour, when compared to its fruit counterpart, experiences a reduction. The antioxidant activity level in various banana varieties range from 44.4 to 51.6 µg/mL, whereas in Table 1, the antioxidant activity level in Klutuk banana flour is recorded at 27.21% (Siji & Nandini, 2017). This decline is attributed to the 24-hour drying process at a temperature of 50°C during the production of Klutuk banana flour. Notably, temperature serves as a determinant factor that accelerates antioxidant oxidation, resulting in diminished antioxidant activity (Muktisari & Hartati, 2018).

Sugar Content of Sorghum Snack Bars with the Addition of Klutuk Banana Flour

The examination of sugar content reveals that the P3 snack bar formulation attains the highest concentration at 2.86%, while the P1 formulation exhibits the lowest sugar content at 1.94%. Statistical analysis of the sugar content test results indicates a significant impact on the sorghum snack bar formulation with the incorporation of klutuk banana flour (p=0.001), as delineated in Table 2.

As Table 2 delineates, the sugar content in sorghum snack bars incorporating klutuk banana flour ranges from 1.94% to 2.86%. Elevated proportion of klutuk banana flour, coupled with diminished sorghum content in the snack bar formulation, correspondingly yield higher sugar content test results. This phenomenon stems from the inherently higher sugar content in Klutuk banana flour, specifically at 2.78%, surpassing that in sorghum. The recorded sugar content of 2.86 gram per 100 gram in sorghum snack bars aligns with the general benchmark for snack bars

containing less than 9 gram of sugar, as stipulated by Musita (2014). However, it exceeds the criteria outlined in SNI 01-3702-1995 for diabetes diet biscuits, which mandates a maximum sugar content of 1%. The elevation in sugar content is attributed to the heating process, resulting in reduced water content and a subsequent rise in the percentage of sugar content (Sutrisno & Susanto, 2014).

Crude Fiber Content of Sorghum Snack Bars with the Addition of Klutuk Banana Flour

The assessment of crude fiber content reveals that the P3 snack bar formulation exhibits the highest concentration at 70.89%, whereas the P1 formulation displays the lowest crude fiber content at 46.04%. Statistically, a highly significant influence on the sorghum snack bar formulation with the incorporation of klutuk banana flour on crude fiber content is evident (p<0.001), a trend outlined comprehensively in Table 2.

It is noteworthy that the elevated crude fiber content in Klutuk banana flour relative to sorghum underpins the observed statistical significance. Diabetes mellitus patients are recommended to intake 20-35 gram of fiber daily (Indonesia Endocrinology Association, 2019). The fiber content per unit of sorghum snack bar, with the addition of klutuk banana flour, ranges from 11.51 to 17.72 gram. Consequently, each serving of sorghum snack bar incorporating klutuk banana flour satisfactorily meets the daily crude fiber requirements, spanning from 46.04% to 70.88%. These findings position sorghum snack bars with klutuk banana flour as a viable dietary option, contributing more than 10% of the daily fiber intake and aligning with the classification of high-fiber snacks (Indrastati & Anjani, 2016). Crucially, the documented fiber content in sorghum snack

bars incorporating klutuk banana flour aligns with the guidelines established by the USDA (2018) for Nutri-Grain Fruit and Nut Bars, specifying a minimum requirement of 7.5 gram of crude fiber content.

Individual with diabetes mellitus can regulate their blood glucose level through the consumption of foods rich in fiber. This dietary approach proves effective by retarding gastric emptying and abbreviating intestinal transit time, consequently mitigating glucose absorption and contributing to reduced blood glucose levels (Avianty & Ayustaningwarno, 2013).

Antioxidant Activity of Sorghum Snack Bars with the Addition of Klutuk Banana Flour

Results of the antioxidant activity assessment revealed that the P3 formulation exhibited the highest levels at 15.05%, whereas the P1 formulation displayed the lowest antioxidant activity at 9.06%. This discrepancy is attributed to the inherent antioxidant content in raw sorghum seeds, which registers at 46.5%, whereas Klutuk banana flour demonstrates an antioxidant activity of 27.21%. The primary sources of antioxidants in sorghum seeds encompass anthocyanins, tannins, and phytic acid (Suarni & Firmansyah, 2016). Additionally, Linda & Panunggal (2015) identify flavonoids, total phenols, and antioxidant activity as key antioxidant sources in sorghum. Statistically, the analysis underscores a significant impact of the sorghum snack bar formulation with the inclusion of klutuk banana flour on antioxidant activity levels ($p < 0.001$). Detailed results of the antioxidant activity level test are presented in Table 2.

The diminished antioxidant activity observed in snack bars compared to the raw materials before their production is attributed to the processing steps involved in snack bar manufacturing, which includes both the heating process during pop sorghum production and baking in ovens. The conventional popping method employed in producing sorghum pop leads to a reduction in antioxidant activity levels in the resulting snack bars (Linda & Panunggal, 2015), with temperatures ranging from 170°C to 200°C during the popping process (Salsabiela et al., 2021). Notably, antioxidant activity experiences a

decrease of approximately 20% when subjected to a temperature of 90°C (Hidayati, 2017).

In accordance with Linda & Panunggal (2015), employing the extrusion method, showcased an antioxidant activity level of 64.431%. Nevertheless, the subsequent baking phase in an oven at 130°C, integral to the production of snack bars, could potentially accelerate antioxidant oxidation, resulting in a subsequent reduction in antioxidant activity (Hastuti & Rustanti, 2014). Additionally, the washing step in the creation of pops holds the capacity to dissolve water-soluble antioxidant compounds, including flavonoids like anthocyanins (Reis et al., 2018). It is noteworthy that bananas, a component in the snack bar formulation, contribute to antioxidant activity due to their content of vitamin C and vitamin E, both recognized antioxidants (Khoozani et al., 2019). Vitamin E, or tocopherol, particularly exhibit notable resistance to heat (Pambudi et al., 2009).

The classification of antioxidant activity level is determined based on percentage value, where a percentage greater than 50% signifies high antioxidant activity, a range of 20-50% indicates moderate antioxidant activity and a percentage less than 20% indicates low antioxidant activity whereas an antioxidant activity level of 0% for DPPH radicals signifies a lack of reduction in these radicals (Wulansari & Chairul, 2011). In the specific context of sorghum snack bar formulations with the addition of Klutuk banana flour, each formulation exhibit a low antioxidant activity, as evidenced by an aggregated antioxidant activity level falling below the 20% threshold. This classification concurs with established criteria. It is crucial to underscore that for individuals with diabetes mellitus, the supply of dietary sources abundant in antioxidants holds paramount significance. This dietary strategy is essential for impeding the production of free radicals, thereby mitigating oxidative stress and preventing vascular complications commonly associated with diabetes (Prawitasari, 2019).

Sensory Characteristics of Sorghum Snack Bars with the Addition of Klutuk Banana Flour

The sensory characteristics in sorghum snack bars with the incorporation of Klutuk banana flour was assessed through organoleptic analysis employing hedonic tests. This sensory assessment involved a cohort of 20 semi-trained panelists, specifically selected from students enrolled in the Undergraduate Nutrition Study Program and Food Technology Bachelor Study Program at Muhammadiyah University of Semarang. The chosen panelists possessed relevant expertise, having undergone the Food Technology Science course as part of their academic curriculum.

a. Sorghum Snack Bar Texture with the Addition of Klutuk Banana Flour

The mean panelists’ preference score for the texture attribute of sorghum snack bars enriched with Klutuk banana flour ranged from 2.55 to 3.10. Notably, the highest average value was recorded in the P3 formulation, indicating a favorable liking level on the “like moderately” scale. In contrast, the lowest average was observed in the P1 formulation, registering on the “like slightly” scale. The discernible escalation in average texture values across formulations suggests a positive correlation with the increasing proportion of Klutuk banana flour. This implies that the greater the addition of Klutuk banana flour, the more favorably the panelists evaluated the texture. Detailed results of the texture tests for sorghum snack bars with Klutuk banana flour incorporation are tabulated in Table 3.

Statistically, the analysis reveals a non-significant effect on the texture of sorghum snack

bars with the incorporation of Klutuk banana flour (p=0.241). This finding can be attributed to the inherent firm texture of sorghum resulting from the puffing process (pops). As elucidated in the study by Salsabiela et al. (2021), sorghum inherently possesses a hard texture, necessitating additional processing and the inclusion of supplementary ingredients to enhance the characteristics of the snack bar. However, notably, in the present investigation, the introduction of Klutuk banana flour did not yield a statistically significant impact on the texture of sorghum snack bars.

b. Sorghum Snack Bar Color with the Addition of Klutuk Banana Flour

The mean panelists’ preference scores for the color attribute of sorghum snack bars enriched with Klutuk banana flour varied between 2.60 and 3.10. Notably, the highest average value was observed in the P3 formulation, indicative of a liking level characterized as “like moderately” on the sensory scale. Conversely, the lowest average was recorded in the P1 formulation, aligning with a “like slightly” level. The discernible elevation in average color values across formulations suggests a positive correlation with the increasing incorporation of Klutuk banana flour. This implies that the augmented addition of Klutuk banana flour corresponds to an enhanced preference among panelists. Detailed results of the color tests for sorghum snack bars with Klutuk banana flour incorporation are presented in Table 3.

Statistically, the analysis indicates a non-significant effect on the color of sorghum snack bars with the addition of Klutuk banana flour (p=0.250). Across all formulations, the color of the

Table 3. Sensory Characteristics of Sorghum Snack Bars with the Addition of Klutuk Banana Flour

Formulation	Texture	Color	Flavor	Aroma	Mean
	Mean ± Standard Deviation				
P0	2.45±0.82	2.60±1.04	2.80±0.95	2.75±0.91	2.65
P1	2.55±0.60	2.75±0.91	2.70±0.97	2.85±0.87	2.70
P2	2.70±0.80	2.95±0.68	2.50±0.82	2.95±0.94	2.77
P3	3.10±1.02	3.10±0.85	2.40±1.04	3.00±1.07	2.90

Note: Formulation of Sorghum:Klutuk banana flour
P0 = (100%:0%), P1 = (90%:10%), P2 = (80%:20%), P3 = (70%:30%)
Hedonic scale = 5 : Like extremely, 4 : Like moderately, 3 : Like slightly, 2 : Neither like, 1 : Dislike

snack bar exhibits a consistent hue, characterized as brown to yellowish-brown. This uniformity in color results from the oven baking process, inducing a brownish-yellow to shiny brown appearance, primarily attributed to the Maillard reaction (Salsabiela et al., 2021). The Maillard reaction, prompted by the addition of sugar and accompanied by a heating process, contributes to the rapid development of a brownish color (Salsabiela et al., 2021). Notably, the progressive addition of Klutuk banana flour correlates with an intensified brownish-yellow coloration in the sorghum snack bar.

c. Sorghum Snack Bar Flavor with the Addition of Klutuk Banana Flour

The mean panelists' taste preferences for sorghum snack bars enriched with Klutuk banana flour spanned from 2.40 to 2.70, with all formulations falling within the "like slightly" range on the sensory scale. The observed decline in the average taste scores across formulations is postulated to stem from an increased disfavor among panelists corresponding to the augmented addition of Klutuk banana flour. Detailed results of the taste tests for sorghum snack bars with Klutuk banana flour incorporation are delineated in Table 3.

Statistically, the analysis reveals a non-significant effect on the taste of sorghum snack bars with the inclusion of Klutuk banana flour ($p=0.338$). This observation is attributed to the incorporation of supplementary ingredients in the sorghum snack bars with Klutuk banana flour, specifically sugar and skim milk flour in equivalent amounts, contributing to an overall sweet taste profile. Notably, the incremental addition of Klutuk banana flour corresponds to a reduction in flavor intensity, as Klutuk banana flour inherently imparts a bland taste (Musita, 2014).

d. Sorghum Snack Bar Aroma with the Addition of Klutuk Banana Flour

The mean panelists' taste preferences for sorghum snack bars with the incorporation of Klutuk banana flour ranged from 2.75 to 3.00.

Significantly, the P3 formulation achieved the highest average value, denoting a liking level on the "like" scale, while the lowest average was registered in the P1 formulation, indicating a liking level on the "somewhat like" scale. This observed increase in average taste scores across formulations is postulated to be associated with the progressive addition of Klutuk banana flour, suggesting a positive correlation between increased Klutuk banana flour content and enhanced liking among panelists. Detailed results of the taste test for sorghum snack bars with Klutuk banana flour incorporation are outlined in Table 3.

Statistically, the analysis indicates a non-significant effect on the aroma of sorghum snack bars with the inclusion of Klutuk banana flour ($p=0.472$). This outcome can be attributed to the inherent lack of a distinctive aroma in sorghum itself (Lestari & Kristiastuti, 2016). The aromatic profile of sorghum snack bars enriched with Klutuk banana flour is primarily shaped by the influence of high temperatures during the heating process and the inclusion of sugar, which harbors furaneol compounds known for producing a sweet and volatile aroma (Salsabiela et al., 2021). Furaneol compounds are generated through the Maillard reaction, originating from 2-hydroxy propanal (Waskito et al., 2014). The Maillard reaction plays a pivotal role in aroma and taste development in food products, providing a foundation for the aroma noted in sorghum snack bars with the addition of Klutuk banana flour, falling within the range of slightly liked to liked by the panelists.

The Best Formulation of Sorghum Snack Bar with the Addition of Klutuk Banana Flour

As depicted in Table 3, the panelists' average scores for sensory characteristics, encompassing texture, color, taste, and aroma, reveal that the sorghum snack bar with the inclusion of Klutuk banana flour garnered the highest acceptance amongst panelists in the P3 formulation.

CONCLUSION

Sorghum snack bars enriched with Klutuk banana flour represent a targeted development aimed at catering to the dietary preferences of individuals afflicted with diabetes mellitus. Within

the formulations, P3 stands out with elevated levels of sugar, crude fiber, and antioxidant activity, albeit the latter remaining relatively modest. The research findings underscore the discernible impact of incorporating Klutuk banana flour on sugar content, crude fiber, and antioxidant activity in sorghum snack bars. In evaluating sensory characteristics, encompassing texture, color, taste, and aroma, it is notable that the addition of Klutuk banana flour exhibits no statistically significant effects. However, among the formulations, P3 emerges as the most favored by panelists. This preference aligns with the overall acceptability of the sorghum snack bars with Klutuk banana flour.

As a prudent recommendation, the daily consumption of sorghum snack bars with Klutuk banana flour is advised at two portions. This suggestion is derived from a comprehensive assessment of nutritional components and sensory attributes, emphasizing P3 as the optimal formulation in the context of both sensory preference and nutritional content for individuals managing diabetes mellitus.

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SENSORY ATTRIBUTE PROPERTIES AND SHELF LIFE OF CHICKEN-HERBAL ESSENCE FUNCTIONAL DRINKS

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ABSTRACT

Chicken-herbal essence, a nutritionally rich functional beverage boasting elevated levels of proteins and amino acids, is renowned for its health-promoting attributes, notably its positive effects on lactation. Comprising an amalgamation of free-range chicken, red ginger, brown sugar, honey, sesame oil, nutmeg, salt, and water, this elixir undergoes a discernible deterioration in quality when subjected to prolonged storage. Consequently, imperative research endeavors are undertaken to elucidate the temporal constraints of storage for this esteemed functional drink. The present study is oriented towards gauging the degradation in quality and prognosticating the shelf life of chicken-herbal essence when exposed to three distinct temperatures—refrigerator temperature (10°C), ambient room temperature (25–27°C), and an elevated temperature (35°C)—utilizing the Arrhenius method as the analytical framework. Conducted over a rigorously observed four-week duration at both the Djuanda University Food Laboratory and the LPPOM MUI Laboratory, this study systematically examines diverse parameters, including pH levels, total dissolved solids, total plate count (TPC), and a comprehensive sensory evaluation. The pH testing, conducted employing a precision pH meter, yields a range of 4.676 – 5.074, while total dissolved solids ranged from 27,100 – 27,450 Brix; total plate count ranged from 0 – 20 CFU/mL. In the sensory test, samples at three temperatures were withdrawn upon panelists' arrival. Generally, refrigerated and room temperature storage showed higher acceptability than higher temperature (35°C) storage. Shelf life estimation indicated that refrigerated storage (10°C) extended to 53 days, surpassing the other temperatures.

Keywords: chickens, food shelf life, functional food, spices

INTRODUCTION

Originating from Asian culinary traditions, Chicken Essence is a revered elixir crafted from concentrated chicken meat extract. Its protein-rich profile endows it with cultural significance, especially for convalescents, expectant mothers, and lactating women in Asian societies (Chao et al., 2004). This elixir confers numerous nutritional benefits, including heightened protein intake, restoration of physical vigor, fatigue alleviation, and enhanced cognitive functions such as memory and concentration in educational pursuits (Li et al., 2012). A contemporary iteration, Chicken-Herbal Essence, fuses chicken extract with herbal elements. Notable ingredients like red ginger, palm or coconut sugar, nutmeg, sesame oil, and trigona honey synergistically contribute to the formulation of this nutritive elixir (Sulaeman et al., 2022).

Food product packaging mandates the inclusion of shelf life information, a critical facet linked to both product safety and the assurance

of delivering quality to consumers (Kusnandar et al., 2010). However, a pertinent challenge in ascertaining shelf life is the temporal constraint. Therefore, there arises a necessity for a rapid, cost-effective method capable of approximating actual shelf life with precision (Herawati, 2008).

Estimating shelf life often involves implementing the Accelerated Shelf-Life Testing (ASLT) method, wherein food products undergo storage in an environment conducive to expedited deterioration. This method induces rapid damage by elevating temperature or humidity conditions beyond those encountered in actual storage conditions (Kusnandar 2010). A specific technique employed in research, the Arrhenius model (Suwita et al., 2017), is integral to ASLT.

The functional beverage known as chicken-herbal essence undergoes a quality decline with prolonged storage. In light of this, the research endeavors to elucidate the trajectory of this decline and estimate the shelf life of chicken-herbal essence when subjected to three distinct

temperatures: refrigeration (10°C), ambient room conditions (25-27°C), and elevated temperature (35°C). The application of the Arrhenius method is instrumental in this investigation, offering a systematic approach to comprehend the kinetics of quality deterioration under varied thermal conditions.

METHODS

Materials and Tools

The chicken-herbal essence, a functional food product derived from chicken, utilized free-range chicken carcasses aged at 3 months from poultry farms in the Pabuaran region, Bogor Regency. Additionally, the formulation incorporated red ginger (*Zingiber rubrum var roscoe*), nutmeg (*Myristica fragrans Houtt.*), sesame oil, salt, and coconut sugar sourced from traditional markets in Warung Jambu, Bogor City, and Ciawi, Bogor Regency. Trigona honey (bee *Tetragonula bironi*) was supplied by CV Nutrima Sehatalami. Methodologically, a pressure cooker, VWR® pH1100 H-pH meter, hand-refractometer, and standard laboratory equipment were deployed for pH assessment, total plate count, total dissolved solids measurement, and sensory evaluation, respectively.

Chicken Essence Production

The formulation of Chicken-Herbal essence followed the adapted methodology from Sulaeman et al. (2022), employing a double-boiled cooking technique with a pressure cooker set at 100°C for a duration of 4 hours. The primary ingredient comprised free-range chicken, complemented by red ginger, palm sugar or coconut sugar, sesame oil, nutmeg, salt, and mineral water. Subsequent to ingredient amalgamation, excluding honey, a meticulous stirring process ensued to achieve uniformity before transferring the mixture to a small saucepan. Within this receptacle, a strategically placed inverted glass bowl facilitated the separation of chicken essence from residue during the cooking process, easing subsequent filtration. Following the cooking phase, meticulous filtration ensues to segregate residue from the essence. The incorporation of *Trigona spp* honey

followed, with subsequent separation of fat and pasteurization at 80°C for 10 minutes, ensuring the formulation adheres to rigorous nutritional standards.

The Sensory Attributes Test

The sensory evaluation employed in this study adopts a hedonic approach, conducted weekly through a comparative analysis between freshly produced and stored chicken-herbal essence samples. A group of 30 semi-trained panelists participates in this evaluative process, assessing the sensory attributes, encompassing color/appearance, viscosity, smell/aroma, taste, and overall acceptability of the product (Meilgaard, Carr, & Civille, 1999). Each panelist evaluated samples at their individual tables, with specimens from three different storage temperatures withdrawn simultaneously for unbiased assessment.

Within the context of this sensory evaluation, the stored product was systematically juxtaposed against a control, the latter representing a chicken-herbal essence product crafted prior to the commencement of the sensory analysis. The assessment employed a nuanced scale, ranging from 1 (no longer acceptable) through 7 (same or better than control), offering a spectrum of descriptors that characterize the perceived changes in product quality. The scale discerned gradations of receptivity loss, from very obvious to subtle differences in comparison to the control. The scales used in this sensory test were: 1 (no longer acceptable); 2 (very obvious loss of acceptability); 3 (more obvious loss of acceptability); 4 (starting to lose acceptability); 5 (more obvious differences but still acceptable); 6 (slightly different from control) and; 7 (same or better than control).

The pH Test

The pH analysis was conducted weekly, adhering to standardized laboratory procedures as outlined in the Operating Manual VWR® pH1100. The protocol steps were: initial rinsing and cleaning of the pH electrode with distilled water and tissue, gradual immersion of the electrode into the sample, utilization of provided buffer solutions for probe stability, activation of pH or mV display via the **MODE** button, and the reading of pH

values displayed on the screen within the Stability Control Range ± 15 seconds.

The Total Dissolved Solids Test (TDS)

The assessment of total dissolved solids adhered to standardized laboratory procedures utilizing a hand refractometer. Scheduled evaluations were conducted at specific intervals, including week 0 (day 2), weeks 1, 2, 3, and 4, across all treatments and three distinct temperature conditions.

Total dissolved solids (TDS) were gauged utilizing a hand-refractometer. The procedural steps included the initial rinsing of the refractometer prism with distilled water, followed by gentle wiping with a soft cloth. Subsequently, a precise quantity of chicken-herbal essence was applied to the refractometer prism, and the Brix degree, a measure of total dissolved solids, was determined with reference to established guidelines (Wahyudi & Dewi, 2017).

The Total Microbial Test (SNI ISO 4833-1:2015)

The microbiological assessment of chicken-herbal essence was conducted weekly over a four-week period employing the Total Plate Count (TPC) method. A specific volume of the liquid test sample was introduced into an empty Petri dish and homogenously mixed with a liquid agar culture medium to generate a pouring plate. The medium of choice for this investigation was PCA (Plate Count Agar), a well-established substrate for microbial growth.

A parallel plate was prepared following identical parameters, employing decimal dilutions of the test sample or the initial suspension. Subsequently, the prepared plate underwent incubation in aerobic conditions at 30°C for a duration of 72 hours. Post-incubation, microbial counts were systematically conducted utilizing a colony counter. The quantification of microorganisms per gram or per milliliter of the test sample ensued through a rigorous calculation derived from the enumeration of colonies present on plates containing fewer than 300 colonies.

Estimating the Shelf Life of Chicken-Herbal Essence

The determination of shelf life in this experimental inquiry was conducted through the application of the Arrhenius equation. The constant representing the rate of quality degradation at three distinct storage temperatures was subsequently integrated into the Arrhenius equation, articulated as follows:

$$\ln k = \ln k_0 - E/RT.$$

The initial step involved establishing the correlation between the natural logarithm of the rate constant ($\ln k$) and the reciprocal of the absolute temperature ($1/T$), utilizing temperature values expressed in Kelvin units.

RESULTS AND DISCUSSION

The pH Test Results

Figure 1 illustrates the average pH trajectory of Chicken-Herb essence during a 4-week storage period. A discernible correlation emerged between the pH test results and the microbial count, indicating a positive association.

Notably, the sample with the highest pH, specifically pH 5.047, exhibited the greatest microbial presence. The inclusion of trigona honey in this study, renowned for its sweet and sour taste with a pH value of 4 (Devianti, Soetarto, & Hendarto, 2015), significantly impacted the overall

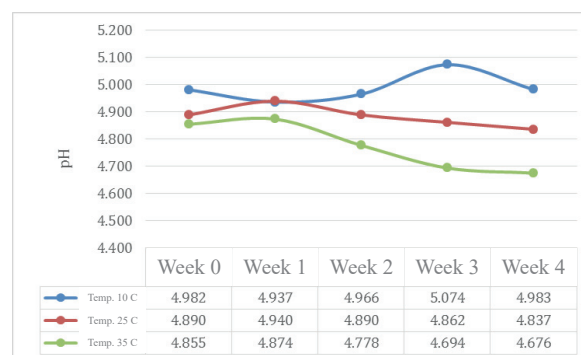


Figure 1. The average pH of Chicken-Herb essence during 4 weeks of storage at 3 different temperatures

pH of the chicken essence product, stabilizing it within the range of 4.8 – 4.9. The complex interplay of enzymatic reactions during storage is a pivotal factor influencing pH alterations, as enzymes catalyze chemical reactions leading to diverse changes in food composition (Suwita et al., 2017). Examining Figure 1, it is evident that when stored at a temperature of 10°C, there was a discernible pH increase during the 2nd and 3rd weeks, succeeded by a decline in pH by the 4th week. Conversely, Chicken Essence stored at temperatures of 25°C and 35°C exhibited a prevailing tendency towards pH reduction. This trend aligns with the findings of Rusli et al. (2022), wherein pH measurements in ginger latte drink products showcased a consistent negative trend, indicative of product compromise due to microbial fermentation activities. Microbial fermentation generates alcohol (ethanol), CO2 gas, and organic acids, contributing to heightened acidity and sourness in the product, substantiated by a concurrent decrease in pH values (Anagari et al., 2011).

The occurrence of protein denaturation can induce a shift in the product’s pH towards alkalinity. Globular proteins, in particular, are prone to denaturation, resulting in alterations to their molecular composition and subsequent changes in both physical and physiological properties (Winarno, 2004). However, the observed pH shifts in the results of this investigation were relatively modest in magnitude.

The Total Plate Count (TPC) Results

As depicted in Table 1 the average count of microorganisms in Chicken-Herb Essence over a 4-week storage period is presented. Table 1 details that the microbial growth observed during storage falls within the range of 0 – 20 CFU/mL. Adhering to the regulatory guidelines outlined in

BPOM Regulation No. 13 of 2019, the acceptable microbial limit is stipulated at 10⁴ colonies/g, with a maximum threshold for microbial contamination in heat-treated ground and processed meat, poultry, and game meat categories set at 10⁶ colonies/g. Notably, the findings underscore that the storage of Chicken-Herb essence over the 4-week duration remains well below the established limits for microbial contamination, as per regulatory standards.

An observable correlation emerges from the pH test results and the microbial counts, revealing a discernible pattern. Specifically, the sample exhibiting the highest pH, recorded at 5.047, corresponds to the highest microbial presence among the samples.

The Total Plate Count (TPC) examination adheres to the standards outlined in SNI ISO 4833-1:2015. Various groups of microorganisms exhibit distinct optimal, minimal, and maximal pH ranges conducive to their growth. Bacteria typically thrive in the pH range of 6.0 – 8.0, while yeast favors the pH range of 4.5 – 6.0, and fungi flourish in the pH range of 3.5 – 4.0. An intrinsic quality of food lies in its buffering capacity, signifying its ability to withstand pH fluctuations. Foods with low buffer capacity are susceptible to rapid pH alterations in response to acidic or alkaline byproducts generated by microorganisms. Conversely, foods with high buffer capacity demonstrate greater resilience to such pH changes (Khutami, Sumiwi, Ikram, & Muchtaridi, 2022; Asiah, Cempaka, & David, 2018). Figure 2 visually depicts the correlation between storage duration and the total count of microorganisms. A discernible pattern emerges, illustrating a relationship between the pH test outcomes and the microbial population. Specifically, the sample exhibiting the highest pH value, recorded at 5.047, corresponds to the highest microbial presence among the samples.

Table 1. Average Microorganisms Count of Chicken-Herb Essence During 4 Weeks of Storage

Temperature	Time				
	Week 0 (CFU/mL)	Week 1 (CFU/mL)	Week 2 (CFU/mL)	Week 3 (CFU/mL)	Week 4 (CFU/mL)
100C	17.5	5	5	20	5
250C	5	5	0	10	0
350C	10	10	5	0	5

The Total Dissolved Solids (TDS) Test Results

The results derived from the analysis of total dissolved solids (TDS) offer valuable insights into the intrinsic sugar content of the constituent ingredients. The investigation underscores a nuanced pattern, demonstrating nominal fluctuations in the total dissolved solids value throughout the storage period. A minimal decline in TDS values during storage signifies that microbial utilization of sugar is limited, suggesting a restrained microbial presence in the beverage (Kusumawati, 2008). This observation is consistent with the findings obtained from the Total Plate Count (TPC) test conducted in this study, revealing a diminished total microorganism count. Collectively, these findings converge to signify a state of minimal food deterioration, further substantiated by the restrained reduction observed in the total dissolved solids value.

Shelf Life Estimation of Chicken-Herbal Essence

Utilizing the principles of the Arrhenius equation, the data for estimating the shelf life of Chicken-Herbal essence products has been derived, as illustrated in the presented Table 4.

Table 4 delineates the shelf life estimation data, stratified into two comprehensive categories: weeks and days. This dual classification is integral to elucidating the potential impact of escalating storage temperatures on the expeditious degradation of product quality within abbreviated temporal intervals. The results within Table 4 elucidate a

conspicuous pattern, indicating a notable inverse relationship between higher storage temperatures and reduced estimated shelf life durations. This observed trend harmonizes with the research conducted by Rusli et al. (2022), wherein the storage of ginger latte products at 45°C exhibited a discernibly hastened rate of deterioration compared to the lower storage temperature of 35°C.

The examination of two pivotal parameters, pH and TPN, as delineated in Table 4, elucidates a distinctive pattern wherein the pH parameter exhibits a more rapid decline, signifying a swifter compromise in shelf life quality. This observed phenomenon may be attributed to enzymatic reactions inherent in the product. Consequently, applying the Arrhenius model to estimate the shelf life of Chicken-Herb essence across three distinct temperatures reveals respective durations of 7.5, 3.8, and 4.6 weeks.

In contrast, the TPN parameters suggest a relatively prolonged estimated shelf life. This disparity can be attributed to a post-packaging pasteurization process aimed at eliminating potential microbial contaminants introduced during the packaging phase, contributing to an enhanced preservation of product integrity.

Sensory Attributes Test Results

Sensory evaluations were conducted on the chicken-herbal essence product following a storage duration of 6 weeks. The primary objective of this assessment was to ascertain the acceptability of the chicken-herbal essence among panelists after

Table 2. Total Dissolved Solids of Chicken-Herb Essence in 4 Weeks Storage

Time Temp.	Week 0 (%Brix)	Week 1 (%Brix)	Week 2 (%Brix)	Week 3 (%Brix)	Week 4 (%Brix)
10°C	27,450	27,270	27,370	27,230	27,270
25°C	27,350	27,310	27,150	27,310	27,270
35°C	27,383	27,383	27,100	27,383	27,310

Table 4. Shelf Life Estimation of Chicken-Herbal Essence using the Arrhenius Model

Parameter	Shelf life (weeks)			Shelf life (days)		
	10°C	25°C	35°C	10°C	25°C	35°C
pH	7,5268	3,8825	4,6119	52,6877	27,1776	32,2834
TPN	19,8285	9,5951	7,8852	138,7996	67,1656	55,1963

Note: TPN=Total Plate Numbers

the specified storage period. The ensuing outcomes derived from the sensory test are comprehensively presented in the subsequent table for detailed examination and analysis.

As delineated in the table, panelists consistently rated the acceptability of chicken-herbal essence from 5.3 to 6.1 on the scale from the first to the fourth week, maintaining overall acceptance with marginal variations. However, from the fifth week onwards, a more noticeable decline in acceptability surfaced, though not universally across all products. Consequently, storing chicken-herbal essence for six weeks remains generally acceptable, albeit with a perceptible reduction in overall acceptability during the latter weeks of storage.

Analyzing the scale range in the sensory test, the initiation of acceptability decline is discerned at a rating of 4. Utilizing overall acceptability as the basis, calculations were conducted to determine the onset of shelf life deterioration through regression analysis of this dataset. The resultant regression equation indicates that the shelf life, when stored in an open room at a temperature of 30°C based on sensory assessments, is estimated to be 22.935 weeks or approximately 22 weeks, as illustrated in Figure 2.

This research holds the merit of employing the Arrhenius model for accurate shelf life estimation, providing proximity to actual shelf life values. The utilization of this model stands out for its efficiency, reducing both time and cost implications in comparison to conventional methods involving actual storage conditions for shelf life determination.

The choice of pasteurization as the sterilization method in this study presents an avenue for exploring alternative sterilization techniques with potential extensions to shelf life. Additionally, it is noteworthy that not all parameters for shelf life estimation were comprehensively addressed in this research.

CONCLUSION

Chicken-herbal essence underwent storage at three distinct temperatures, revealing a notable decline in quality across the stored samples. Utilizing the Arrhenius model, the estimated shelf life for chicken-herbal essence stored at 25°C is calculated to be approximately 3.88 weeks, indicative of the temporal span before a significant degradation in product attributes is anticipated. The implications drawn from this research underscore the necessity for further investigations incorporating additional parameters, notably water content. Moreover, the study recommends the

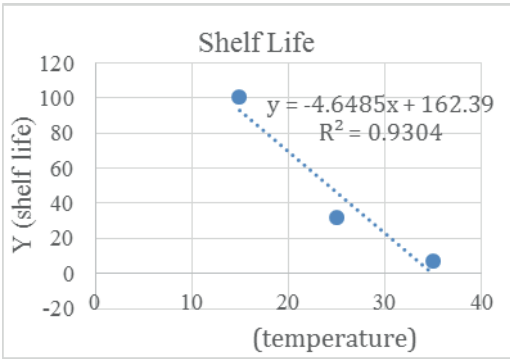


Figure 2. Shelf Life Estimation based on Sensory Test

Tabel 6. Sensory Attributes Property test of Chicken-herbal Essence in 6 weeks of storage

Week -	Color /Visual			Texture / Thickness			Aroma			Taste			Overall Acceptance		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1	6.3	6.0	5.9	6.1	6.2	6.1	5.9	6.1	5.7	6.1	6.0	5.9	6.1	6.0	5.9
2	5.7	6.4	5.6	5.9	6.1	5.9	5.5	6.0	5.9	5.7	6.0	5.7	5.8	6.1	5.8
3	6.2	6.2	5.6	6.2	6.2	5.6	5.8	5.9	5.0	5.8	6.2	4.9	6.0	6.1	5.3
4	6.1	6.2	6.1	6.0	5.8	5.9	5.5	5.6	5.5	5.5	5.4	5.5	5.8	5.6	5.7
5	5.4	5.1	6.2	5.8	5.3	5.9	5.4	3.6	5.4	5.3	3.2	5.3	5.2	3.9	5.6
6	5.3	6.1	5.1	5.7	6.0	5.1	5.6	5.9	3.0	5.4	5.7	3.1	5.5	6.0	3.5

Note: A = Chicken-herbal essence product stored at 15°C, B = Chicken-herbal essence product stored at 25-27°C, C = Chicken-herbal essence product stored at 35°C

exploration of alternative methods for estimating shelf life.

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THE POTENTIAL OF SUNFLOWER SEED BISCUITS IN LOWERING BLOOD GLUCOSE AND MALONDIALDEHYDE LEVEL IN TYPE 2 DIABETES MELLITUS RAT MODELS

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is marked by early hyperglycemic symptoms and concurrent insulin resistance, leading to insulin secretion dysregulation. This resistance correlates with heightened Reactive Oxygen Species (ROS) level and reduced malondialdehyde. T2DM elevates malondialdehyde, necessitating antioxidant-rich interventions. Sunflower seed biscuits serve as a rich source of enzymatic antioxidants. The primary objective of this investigation was to substantiate the capacity of sunflower seed biscuits to ameliorate blood glucose and malondialdehyde level in T2DM-afflicted rats. Sunflower seed biscuits were investigated for their impact on blood glucose and malondialdehyde in 24 male Wistar rats. Rats were divided into four groups: diabetes rats (K-), simvastatin-treated (K+), receiving 0.72 g sunflower seed biscuits per rat body weight (X1), and 1.44 g (X2). Administered for 28 days via oral gavage, T2DM was induced through a high-fat diet and streptozotocin. Results showed significant blood glucose reduction in treatment groups (X1: 3.99 ng/mL, X2: 2.89 ng/mL) vs. controls (9.8 ng/mL), with statistical significance ($p < 0.05$). Sunflower seed biscuits effectively lowered blood glucose and malondialdehyde in T2DM rats. The X2 group exhibited superior efficacy in reducing both parameters. Thus, sunflower seed biscuits present promise as a viable dietary option for T2DM management.

Keywords: Sunflower seed, diabetes mellitus, blood glucose, malondialdehyde

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a pathological condition delineated by the initial manifestation of hyperglycemic symptoms and a concomitant perturbation in insulin secretion, colloquially referred to as insulin resistance (Perkeni, 2015). The incidence of T2DM frequently correlates with obesity, specifically the augmentation of body weight, which can engender a decrement in the functionality of pancreatic β cells (Al-Sulaiti, et al., 2019). This decrement in insulin sensitivity ultimately precipitates the occurrence of hyperglycemia (Xu, et al., 2019). Hyperglycemic states have the propensity to augment oxidative stress conditions, marked by heightened production of Reactive Oxygen Species (ROS) and serving as a catalyst for elevated free radical generation. The genesis of free radicals within the milieu of T2DM is primarily attributed to the phenomenon of glucose undergoing an auto-oxidative process that surpasses the rate of endogenous antioxidant formation within the organism (Asmat, et al., 2016).

Pathological states that foster an upsurge in free radical level, devoid of commensurate endogenous and exogenous antioxidant reservoirs, have the potential to exacerbate oxidative stress (Asmat, et al., 2016). The proliferation of Reactive Oxygen Species (ROS) within the organism can be reinstated to homeostatic level through the administration of both enzymatic and non-enzymatic antioxidants (Jamuna and Mythili, 2014). Non-enzymatic antioxidants, derived from natural dietary constituents, have demonstrated efficacy in ameliorating the clinical manifestations of diabetes mellitus (Kinasih, et al., 2020; Kurniawan, et al., 2020).

Sunflower seeds represent a natural dietary component that has yet to be fully leveraged. Empirical investigations underscore the substantial presence of essential components within sunflower seeds, including vitamin E, linoleic acid, β -sitosterol, and phenolics, rendering them a valuable reservoir of enzymatic antioxidants (Kiczorowska, et al., 2019; Saini and Sharma, 2013). Sunflower seeds exhibit a notable

phenolic content, quantified at 977.0 mg per 10 gram (Zoumpoulakis, et al., 2017). Beyond their enzymatic antioxidant potential, sunflower seeds have been ascribed additional roles, encompassing anti-inflammatory, antioxidant, and anti-diabetic properties (Zoumpoulakis, et al., 2017).

Sunflower seed biscuits are categorized as functional foods, distinguished by their prolonged shelf stability and enriched complexity in nutrients and bioactive compounds in contrast to unprocessed sunflower seeds (Leverrier et al., 2019). The biscuits' composition comprises 70% sunflower seeds and 30% other constituents. The dosage variants of sunflower seed biscuits administered in this investigation were 0.72 g/day and 1.44 g/day, as previously established in the literature, aimed at attenuating the pro-inflammatory response in diabetic Wistar rats (Mahirdini and Afifah, 2016). This approach aligns with prior research findings (Leverrier et al., 2019; Saini and Sharma, 2013), underscoring its validity in mitigating diabetic-related inflammation.

In accordance with the aforementioned elucidation, the principal objective of this investigative endeavor is to ascertain the therapeutic potential of sunflower seed biscuits (SSB) in mitigating blood glucose level and malondialdehyde concentrations within the context of Type 2 Diabetes Mellitus (T2DM) in Wistar rat models.

METHODS

This study adheres to a rigorous true experimental design, employing a Pre and Post Test with Control Group Design. The analysis of biscuit nutritional composition, encompassing parameters such as energy, protein, fat, carbohydrate, and antioxidant content, was conducted at Chem-Mix Pratama Laboratory in Yogyakarta. Experimental animal husbandry and testing procedures were meticulously executed at the Laboratory of Center for Food and Nutrition Studies (*Pusat Studi Pangan dan Gizi* - PSPG), Universitas Gadjah Mada, Yogyakarta, spanning the period from November 2020 to January 2021. Notably, this research initiative secured the requisite ethical clearance and authorization from the Health Research Ethics Commission at Diponegoro

University, Dr. Kariadi, bearing registration number 113/EC/H/FK-UNDIP/XI/2020.

The determination of the sample size for this experiment adhered to the guidelines established by the World Health Organization (WHO, 2000), specifying a minimum requirement of 5 mice per treatment group for experimental animals. A cohort comprising 24 male Wistar rats, aged between 8 and 12 weeks, and exhibiting body weights ranging from 150 to 200 gram, was procured from the Laboratory of the Center for Food and Nutrition Studies (PSPG), Universitas Gadjah Mada, Yogyakarta, in accordance with Marques et al. (2016). The constituents requisite for biscuit formulation were sourced from a confectionery establishment in Semarang. Streptozotocin (STZ) and Nicotinamide (NA) were acquired from Nacalai Tesque, Japan. The quantification of blood glucose level was executed utilizing the GOD-PAP method, while the measurement of malondialdehyde level was performed employing the ELISA technique in the course of this investigation.

The baseline rat nutrition regimen consisted of Comfeed II, comprising 12% water, 15% crude protein, 3–7% crude lipid, 6% crude fiber, 7% ash, 0.9–1.1% calcium, and 0.6–0.9% phosphorus, administered at a dosage of 20 gram per rat per day. Subsequently, the high-fat feed administered to induce Type 2 Diabetes Mellitus (T2DM) in the rats was comprised of 80% standard feed, 20% pork oil, and 1.5% additional cholesterol, administered at 20 gram per rat per day for a duration of 14 days, followed by ad libitum access to distilled water. Essential experimental equipment encompassed cages, rat feeding troughs, rat drinking bottles, digital scales, and blood sampling apparatus, including micro hematocrit and syringe probes, all procured from the Laboratory of the Center for Food and Nutrition Studies (PSPG), Gadjah Mada University, Yogyakarta.

In this experimental study, the administration of sunflower seed biscuits was meticulously calibrated to ensure equivalency between human and rat dosage. The standard serving size for human snacks, consisted of 40 gram of biscuits, was employed as the benchmark. To establish the corresponding dosage for rats, the sunflower seed content in 100 gram of biscuits was determined

through meticulous calculation. For Dose 1, an allocation of 0.72 gram of biscuits was employed, which correspondingly contained 0.504 gram of sunflower seeds. Within this dosage, the provision of vitamin E amounted to 12.68 mg. In contrast, Dose 2 constituted a 1.44 gram allocation of biscuits, yielding 1.008 gram of sunflower seeds. Within this dosage regimen, the delivery of vitamin E escalated to 25.36 mg. This precise dosing methodology ensures a methodical investigation into the potential effects of sunflower seeds and vitamin E, thereby contributing to the scientific understanding of their impact on rat physiology. Such meticulous dosage calibration is fundamental in the realm of medical research, as it permits accurate interpretation of outcomes and facilitates the derivation of meaningful insights into potential therapeutic or nutritional interventions.

The sunflower seed biscuit preparation commences with meticulous selection of 70 g of dry sunflower seeds and 10 g of oats, both meticulously ground to a homogenous texture via a blender. Subsequently, amalgamate this mixture with 10 g of eggs, 5 g of low-calorie granulated sugar, and 5 g of margarine until uniform. The dough is then flattened into 10 g portions and baked at 130°C for precisely 15 minutes. This meticulous culinary process yields delectable sunflower seed biscuits.

Table 1. Feed Composition of Intervention Rat

Ingredient	Composition (%)
Sunflower seed	70
Oat	10
Chicken egg	10
Low caloric sugar	5
Margarine	5

The induction of type 2 diabetes mellitus (T2DM) in murine models necessitates a meticulously orchestrated protocol. In this endeavor, 24 male Wistar rats were subjected to a systematic regimen. Over the course of one week, these rats were individually housed, receiving a standard daily diet of 20 gram and ad libitum access to distilled water. Ambient temperature was maintained within the range of 28–32°C, complemented by a 12-hours light cycle spanning from 06:00 to 18:00. Rigorous daily monitoring

encompassed food consumption quantification and routine cage sanitation. Prior to commence T2DM induction, it was imperative to establish baseline parameters for each subject. Blood samples were extracted via the retroorbital plexus to ascertain uniform blood glucose and malondialdehyde level. This preliminary screening ensured the health status of all mice at the inception of the study. The subsequent phase of the experiment involved the induction of T2DM in the murine cohort. This was achieved through a 14-days dietary intervention, during which high-fat food was administered at a rate of 20 gram per rat per day (Guo, et al., 2018). Subsequent to Nicotinamide (NA) induction at 110 mg/kg intraperitoneally, Streptozotocin (STZ) was administered intraperitoneally at 45 mg/kg, 15 minutes later. (Ghasemi, et al., 2014; Qasem, et al., 2018). Prior to streptozotocin (STZ) induction, mice were subjected to a fasting regimen. Following 3 days of STZ induction, mice underwent an additional 8-10 hours fast, after which 2 mL of blood was sampled from the retroorbital plexus to assess blood glucose level. Malondialdehyde served as a baseline comparator prior to initiating the intervention involving SFS biscuits. The diagnostic threshold for type 2 diabetes mellitus was established at fasting blood glucose level ≥ 200 g (Li, et al., 2015).

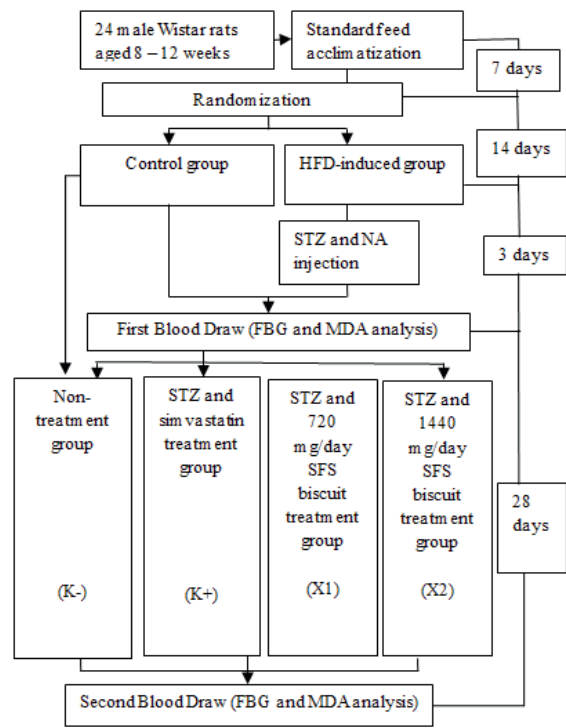


Figure 1. Research Flowchart

In this experimental study, twenty-four rats were stratified into four distinct groups: T2DM rats without any therapeutic intervention (Group K-), T2DM rats administered a daily dosage of 0.18 mg/rat weight/day of simvastatin (Group K+), T2DM rats receiving a daily dose of 0.72 g/rat weight/day of sunflower seed biscuits (Group X1), and T2DM rats subjected to a daily dose of 1.44 g/rat weight/day of sunflower seed biscuits (Group X2). The sunflower seed biscuits were meticulously crushed and transformed into a daily solution, which was freshly prepared each morning. Subsequently, this solution was administered to the T2DM rats via sonde, once daily, in the morning. The intervention involving the sunflower seed biscuit solution in T2DM rats spanned a duration of 28 days, with the rats being concurrently provided with standard food and water throughout the experimental period (Kinasih, *et al.* 2020). Upon study completion, blood samples were collected for conclusive assessment of blood glucose and malondialdehyde concentrations following the intervention.

In this experimental study, data underwent normality assessment via Shapiro-Wilk test. Normal distribution confirmed, enabling the use of paired T test for pre-post comparison. Group distinctions analyzed via *One-Way ANOVA*, followed by *Bonferroni post hoc* tests. *Spearman Rank correlation* explored relationships with $p<0.05$ significance level.

RESULTS AND DISCUSSION

Rats Weights during Experiment

Table 2 illustrates the weight gain observed in the experimental animal groups during the SFS

biscuit administration period. Significant disparities emerged between the pre-test and post-test measurements across all four groups. In the K(+) group, X1, and (K-), a notable decline occurred due to the absence of simvastatin and SFS biscuit intervention in the context of established T2DM. This exacerbated the T2DM condition, intensifying oxidative stress level without antioxidant supplementation. Consequently, these mice exhibited decreased appetite, leading to a decline in body weight (Tabatabaei-Malazy, *et al.* 2017). Pre-intervention, groups exhibited similar mean body weights; post-SFS biscuit administration, all groups showed significant body weight disparities ($p<0.05$).

Injection of streptozotocin in group K(-) mice led to reduced weight loss, attributed to heightened fat decomposition and DNA synthesis inhibition in mammalian enzymes, triggering apoptosis (He, *et al.*, 2015). Insulin resistance can be a factor that induce weight loss in mice by impairing the inhibitory effect of insulin on lipolysis via the cAMP pathway, leading to reduced PKA activation. In the K(-) group, mice exhibited a 21.5% decrease in appetite, translating to an average daily intake reduction of 2 gram. This diminished food consumption contributed to the observed decline in body weight during the study (Fauza, *et al.*, 2019). STZ-induced enzyme inhibition triggers cellular apoptosis, contributing to weight loss in T2DM-afflicted mice. (Nagarchi, *et al.* 2015).

The observed increase in rat body weight in the X2 group closely resembled that in the K(+) group. This suggests that SFS biscuits exhibit comparable efficacy to simvastatin in mitigating oxidative stress, thereby enhancing food intake and influencing body weight parameters. Notably, the

Table 2. Rat Body Weights *Pre* and *Post* Treatment

Treatment Group	Rat Body Weight (gram)			
	<i>Pre</i>	<i>Post</i>	<i>p</i>	Δ
K(-)	202.5±4.7	184.3±4.2	<0.001	18.2±1.5
K(+)	205.2±3.1	231.0±3.3	<0.001	25.8±1.9
X1	203.2±5.2	221.3±4.6	<0.001	18.2±1.2
X2	206.2±3.9	232.2±4.5	<0.001	26.0±1.7
<i>p</i> ¹	*0.435	<0.001		<0.001

Note: *p*= paired T test; *p*¹ = *One-Way ANOVA* test; * = *Kruskal Wallis* test; K(-)= T2DM + non-treatment; K(+)= T2DM + simvastatin; X1= T2DM + sunflower seed biscuit 0.72 g/rat BW/day; X2= T2DM + sunflower seed biscuits 1.44 g/rat BW/day; n= 24 samples

X2 group demonstrated the highest weight gain, likely attributed to the substantial protein content (22.7 gram per 100 gram) in BBM biscuits, which enhanced palatability and appetite in the mice.

Post-Treatment Changes in Blood Glucose and Malondialdehyde Level

The paired T-test analysis presented in Tables 3 and 4 demonstrated a noteworthy decline in blood glucose and malondialdehyde level following the intervention. Additionally, Table 5 illustrated a significant correlation between blood glucose and malondialdehyde after treatment, using doses of 0.72 g/rat body weight/day and 1.44 g/rat body weight/day ($p<0.05$). In this experimental context, SFS biscuits displayed a statistically proven capacity to substantially reduce blood glucose level in T2DM mice. Notably, the X2 group exhibited a markedly superior reduction in blood glucose level compared to the group administered simvastatin K(+). Both X1 and X2 doses displayed significant efficacy in reducing blood glucose level. Remarkably, the effectiveness of the X2 group in reducing blood glucose level rivalled that of simvastatin in T2DM mice. Further

analysis, utilizing Bonferroni’s post hoc test ($p<0.05$), revealed significant differences between treatments in both X1 and X2 (Table 2).

The most significant reduction in blood glucose level was observed within group X2, administered a daily dose of 1.44 g of biscuits, resulting in a value of 95.2 ± 1.9 mg/dL in comparison to group X1. This outcome can be attributed to the heightened antioxidant content within this dosage, along with its rich vitamin E and phenolic composition. Earlier investigations have identified various antioxidant components in sunflower seeds, including phenolic compounds and vitamin E, as reported by Widia et al. (2011) and Kiczorowska et al. (2019). These constituents are recognized for their potential in mitigating blood glucose level. Moreover, the active substances present in pancreatic β cells and the antioxidants found in SFS biscuits contribute to the reduction of malondialdehyde level in T2DM mice, as illustrated in Table 3.

A substantial reduction in malondialdehyde (MDA) level was observed in both treatment cohorts. Mice administered SFS biscuits at a dosage of 1.44 g per rat per day exhibited MDA level closely mirroring those in the group receiving

Table 3. Blood Glucose Level Pre and Post-treatment

Treatment Group	Blood Glucose Level (mg/dl)			
	Pre	Post	p	Δ
K(-)	270.1 \pm 2.1	272.3 \pm 2.2	0.001 ^{abc}	2.1 \pm 0.8
K(+)	268.2 \pm 1.9	89.7 \pm 3.5	<0.001 ^b	178.5 \pm 4.4
X1	269.8 \pm 2.2	135.9 \pm 3.8	<0.001 ^{ac}	133.8 \pm 5.5
X2	268.9 \pm 2.1	95.2 \pm 1.9	<0.001 ^b	173.8 \pm 3.1
p ¹	0.379	<0.001		<0.001

Note: p= paired T test; p¹ = One-Way ANOVA test; ^ap<0.05 post hoc Bonferroni test with group K(+); ^bp<0.05 post hoc Bonferroni test with group X1; ^cp<0.05 post hoc Bonferroni test with group X2; K(-)= T2DM + non-treatment; K(+)= T2DM + simvastasin; X1= T2DM + sunflower seed biscuit 0.72 g/rat BW/day; X2= T2DM + sunflower seed biscuits 1.44 g/rat BW/day; n= 24 samples

Table 4. Malondialdehida Level Pre dan Post-Treatment

Treatment Group	Malondialdehida (ng/dl)			
	Pre	Post	p	Δ
K(-)	9.7 \pm 0.6	9.8 \pm 0.5	0.056 ^{abc}	0.1 \pm 0.1
K(+)	9.8 \pm 0.4	1.8 \pm 0.2	<0.001 ^{bc}	8.0 \pm 0.5
X1	9.8 \pm 0.4	4.0 \pm 0.2	<0.001 ^{ac}	5.8 \pm 0.4
X2	9.7 \pm 0.3	2.9 \pm 0.2	<0.001 ^{ab}	6.9 \pm 0.5
p ¹	0.908	<0.001		<0,001

Note: p= paired T test; p¹ = One-Way ANOVA test; ^ap<0.05 post hoc Bonferroni test with group K(+); ^bp<0.05 post hoc Bonferroni test with group X1; ^cp<0.05 post hoc Bonferroni test with group X2; K(-)= T2DM + non-treatment; K(+)= T2DM + simvastasin; X1= T2DM + sunflower seed biscuit 0.72 g/rat BW/day; X2= T2DM + sunflower seed biscuits 1.44 g/rat BW/day; n= 24 samples.

0.18 mg of simvastatin daily. This investigation underscores the potential equivalence between SFS biscuit-based nutritional intervention and simvastatin pharmaceutical therapy. Notably, a significant contrast emerged between treatment X1 and X2, with the latter yielding the most pronounced MDA reduction at 2.9 ± 0.2 ng/mL, surpassing the former. The administered dosage aligns with established precedents from prior studies, affirming its efficacy in mitigating inflammatory responses in diabetic murine models (Saboori, et al., 2015; Leverrier, et al., 2019; Saini dan Sharma, 2013). Vitamin E in sunflower seeds serves as an antioxidant, augmenting total antioxidant capacity to thwart free radical formation. Furthermore, it mitigates inflammation by inhibiting interleukin-6 (IL-6) release (Septiani, 2018).

Table 4 illustrates a noteworthy Spearman Rank correlation analysis, revealing a significant ($p<0.05$) association between blood glucose and malondialdehyde level. This study reinforces the inverse relationship: decreased blood glucose corresponds to reduced oxidative stress, as indicated by lower malondialdehyde level.

Table 5. Blood Glucose Level and Malondialdehyde Correlation Pre and Post-Treatment

Variable	Pre		Post	
	r	p	r	p
Blood Glucose and Malondialdehyde	-0.062	0.775	0.991	<0.001

This study demonstrates the substantial unsaturated fat content (35.7g/100g) in SFS biscuits, rich in unsaturated fatty acids. Omega-3’s pivotal role includes enhancing insulin sensitivity by mitigating inflammation and elevating adiponectin level (Cheenam, et al., 2019), so this study demonstrates the efficacy of sunflower seeds in reducing blood glucose and malondialdehyde level. Sunflower seed (SFS) biscuits, administered at 0.72 and 1.44 g/weight of mice/day, approximate to 40 and 80 gram or 4 and 8 pieces for humans, prove beneficial as a snack during Type 2 Diabetes Mellitus (T2DM) treatment. Statistical analysis

confirms the significant reduction of blood glucose and malondialdehyde level in T2DM mice through SFS biscuit intervention.

CONCLUSIONS

Sunflower seed biscuits exhibit potential in lowering blood glucose and malondialdehyde level in type 2 diabetes mellitus mice, with optimal effects observed at an intervention dose of 1.44 g/rat body weight/day. SFS biscuits, offering 80g or 8 pieces as a snack, are suggested for T2DM patients, potentially lowering blood glucose and malondialdehyde level.

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FORECASTING LAYER EGG PRODUCTION AS ANIMAL SOURCE FOOD FOR PROTEIN TO REDUCE STUNTING IN WEST JAVA, INDONESIA

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ABSTRACT

Stunting still becomes a global public health challenge affecting millions of people, including Indonesians. To alleviate the situation, Indonesia is fighting to reduce the prevalence of stunting to 10.4% in 2024 by increasing the consumption of Animal Source Foods. This study aimed to forecast the Layer Egg Production in West Java, Indonesia, as an animal source food to provide protein for reducing stunting. The study employed descriptive quantitative and time series approaches using secondary data analysis. The Layer Egg Production for 2023-2025 was forecasted using the Moving Average (MA) and Weighted Moving Average (WMA) using Layer Egg production data for 2000-2022. The WMA method was more accurate as it had a Mean Absolute Percentage Error (MAPE) of 15.32 compared to a MAPE of MA of 16.23. Furthermore, the population projections for West Java 2021-2025 were made by the Geometric method. The study's findings declare that the Layer Egg Production is still low to meet the population demand for West Java. In this case, the population can get 18.77 g of egg per person daily, an average of 2.4g of protein. In addition, the egg contributes 4.2% to the protein required by each person a day.

Keywords: Stunting, Forecasting, Protein, Layer Egg, Production

INTRODUCTION

Childhood stunting is one of the biggest barriers to human growth (WHO, 2014), it affects over 162 million children under the age of five worldwide. A height for age Z- score that is less than two standard deviations of the median of the World Health Organization (WHO) growth charts for child development is referred to as stunting or being too short for one's age (UNICEF, WHO 2018). It is a result of poor nutrition and frequent infections. In addition, it is largely irreversible. Stunting has long-term negative repercussions on people and society, including a decline in cognitive and physical growth, a reduction in productivity (Renyot et al., 2016), poor health, and a higher chance of developing degenerative illnesses such as diabetes (Adair, 2013). Projection shows that 127 million children under five will be stunted in 2025 if trends continue (WHO, 2018). According to the United Nations 2015, the 2030 Agenda for Sustainable Development Goal (SDG) 2 demands for reducing malnutrition in all its forms including stunting, wasting, and micronutrient deficiencies.

According to the Global Nutrition Report 2014, Indonesia has made a lot of effort towards

achieving the stunting target. As a country, Indonesia is fighting to reduce the prevalence of stunting by 10.4% by 2024. Data from the Indonesian Nutrition Status Study (SSGI, 2022) put the prevalence of childhood stunting in Indonesia at 21.6%. In this case, the government of Indonesian is targeting to reduce stunting to 14% by 2024 according to the Presidential Decree Number 72 of 2021 regarding the reduction of stunting. West Java is one of the 12 provinces with the largest cases of stunting at 971.792 cases and a prevalence of 20.2% as shown in Figure 1 that has been named by the Ministry of Health alongside East Nusa Tenggara at 37.8%, West Sumatera at 33.8%, and Aceh at 33.2% (MoH, 2023 ; Yuliastini et al., 2020).

Animal Source Foods (ASF) can assist in the achievement of the nutrition targets endorsed by the World Health Assembly and the Sustainable Development Goals related to reducing stunting and wasting in children under five, as well as obesity and non-communicable diseases (NCDs) in adults (FAO,2022). Animal Source Foods (ASF) are also an essential source of nutrients that offer several benefits for human health (Fernandez, 2022). In this case, animal proteins, including eggs,

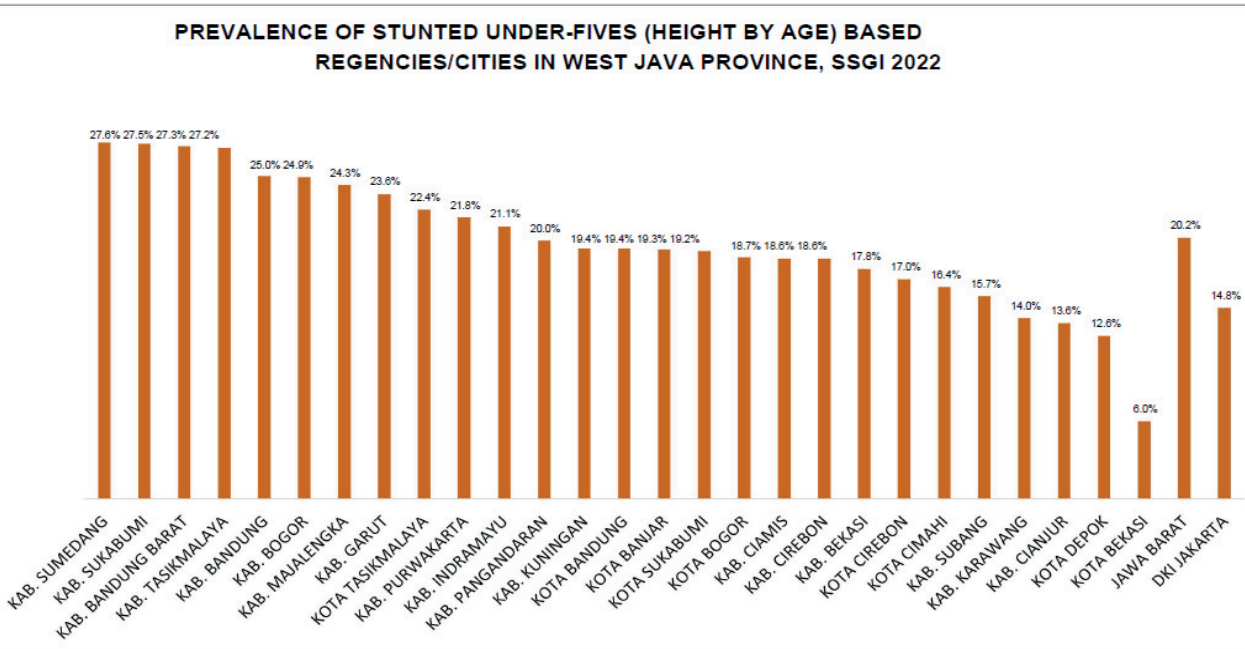


Figure 1. Prevalence of stunted under-five (height by age) based on regencies/cities in West Java Province (Kemenkes, 2021)

meat, poultry products, fish, and dairy products, are considered to have complete proteins (Henchion et al., 2021) since they provide all the essential amino acids required by the human body. Amino acids are the building blocks of proteins and are necessary for various physiological functions, including tissue repair, enzyme production, and hormone synthesis (Adesogan, 2018). Animal source foods are particularly abundant in nutrients such as protein, vitamin B12, iron, and zinc, which can prevent stunting.(Derek Headey, Kalle Hirvonen, 2018) In addition, it also contains omega-3 fatty acids, which are essential for brain function, immune health, red blood cell production, and overall well-being (Khusun, Monsivais, et al, 2022).

Figure 1 shows the rate of stunting in different regencies of West Java with Sumedang as the highest at 27.6%, followed by Sukabumi at 27.5%, Bandung Barat at 27.3%, and Tasikmalaya at 27.2%. Meanwhile, the least prevalence is in Bekasi City at 6.0%. Overall, West Java has a stunting prevalence of 20.2%. West Java is still battling with stunting among children under five. The study aimed to forecast the Layer Egg Production in West Java, Indonesia, as an animal source food to provide protein for reducing stunting.

Table 1. Nutrition Composition of an Egg

Nutrient (Unit)	Whole Egg
Weight (g)	60
Water (percentage)	65-68.5
Calories (Kcal)	70
Protein (g)	6.3
Carbohydrate (g)	0.36
Total fat (g)	4.8
Polyunsaturated fat (g)	1.0
Monounsaturated fat (g)	1.8
Saturated fat (g)	1.6
Cholesterol (mg)	185
Choline (mg)	126
Vitamin A (IU)	270
Vitamin D (IU)	41
Vitamin E (mg)	0.5

METHOD

This study used a descriptive quantitative research method and a time series approach through Secondary Data Analysis. The data used was a time series data of Layer Egg production in West Java during the 2000-2022 period based on Statistics of Indonesia (BPS,2022). It was used for forecasting Layer Egg Production from 2023 to 2025, as shown in Table 3. The population data of West Java for the 2010-2022 period, according to

BPS 2022, was also used. This population data was used in forecasting the population of West Java for the period 2023 to 2025 using the Geometric method, as shown in Table 5. The research data was collected in April-May 2023.

Data Processing and Analysis

The Data was processed using Microsoft Excel. In addition, several forecasting methods were also used for production analysis, which included Moving Average (MA) and Weighted Moving Average (WMA). These methods need sequential data from several years before.

Moving Average (MA) Equation

$$F_t = \frac{A_{t-1} + A_{t-2} + \dots + A_{t-p}}{p}$$

where:

- t = Time (Years)
- F_t = Result of forecast at time t
- A_t = Actual data at time t
- P = Data used for forecast

Weighted Moving Average (WMA) Equation

$$F_t = \sum_{i=1}^p \alpha_i A_{t-i}$$

where:

- t = Time (Years)
- F_t = Result of forecasting at time-t
- A_t = Actual data at time-t
- P = Data used for forecasting.

$$\sum_{i=1}^p \alpha_i = 1, 0 \leq$$

The Mean Absolute Deviation guided the Accuracy of the method used for forecasting (MAD), Mean Square Error (MSE), and the Mean Absolute Percentage Error (MAPE).

1. Mean Absolute Deviation (MAD)

$$MAD = \frac{\sum_{j=p+1}^n |A_j - F_j|}{p}$$

2. Mean Square Error (MSE)

$$MSE = \frac{\sum_{j=p+1}^n |A_j - F_j|^2}{p}$$

3. Mean Percentage Absolute Error (MAPE)

$$MAPE = \frac{\sum_{j=p+1}^n |A_j - F_j| / A_j}{p} \times 100 \%$$

Where

- A_j = Actual Value
- F_j = Forecast value
- P = Number of forecast value

The Geometric method was used

- P_t = Po(1+r)^t
- Po = Initial size
- P_t = Population at time t
- T = Time (years)
- R = Finite population multiplier

Table 2. Layer Egg Production (2000-2022) in West Java

Year	Egg Production
2000	114,279.00
2001	68,050.00
2002	78,945.00
2003	77,634.00
2004	89,349.00
2005	93,472.00
2006	95,143.00
2007	105,361.00
2008	105,046.00
2009	95,627.70
2010	103,427.58
2011	115,787.00
2012	120,123.00
2013	131,586.00
2014	134,581.00
2015	133,435.77
2016	139,192.78
2017	693,379.15
2018	802,859.62
2019	468,872.04
2020	587201.77
2021	661,895.15
2022	699,384.40
Amount	5,714,631.96

Average24,8462.23

RESULTS AND DISCUSSIONS

Based on the data shown in *Table 2*, Layer Egg production had a significant increase in 2017 and later dropped in 2019. The sharp increment can be attributed to the increasing population that creates the demand, increased knowledge regarding the intake of animal protein foods, and an egg-a-day campaign. The decline in layer egg production is noticed in 2019 by 42%. This decrease could be due to the COVID-19 pandemic that distorted many food production systems and the general economy. Forecasting analysis for layer egg production was made using the data in *Table 2* and production for the period 2023 to 2025 was forecasted as shown *Table 3*.

Table 3. Forecasted Layer Egg Production 2023-2025

Year	Projected Layer Egg Production (Tons)
2023	649493.77
2024	680639.77
2025	699384.40
Total	2,029,517.95
Average	676,505.98

The forecasting graph of layer egg production shown in Figure 2 shows that there is a general increase in Egg production using the MA and WMA method over the years. The projected production also showed an increment with the

average production increase in 2023-2025 by 676505.98 tons.

For the two methods used to forecast layer egg production, that is MA and WMA, the method with the lower MAPE can be used to give a more accurate estimate. In this case, WMA at 3 years (3Ft) has got MAPE of 15.32 compared to MA, which has a MAPE of 16.23 at 3Ft. Therefore, the method with the lower error rate is more accurate forecasting method (Suryana & Sukandar, 2022).

Through the use of the population data of West Java from 2016-2020, the population for 2021-2025 was forecasted using the Geometric method as shown in *Table 5*. The findings indicate an increase in the population of West.

As shown in Table 5, the layer egg production has had a steady annual increase. However, the production increased sharply in 2017 until 2018. Furthermore, there was a decline of layer egg production in 2019 by almost half of the previous production. The production later started to rise again in 2020 by 25.2%. The layer Egg production in West Java is fluctuating. This can be attributed to the income, population demand, cost of production of the eggs, and availability of other animal source foods like meat, chicken, and milk (Suryana et al., 2022). Taking average weight of an egg as 60g and 12.8g of protein from 100 grams with 90% as edible portion (Suryana & Sukandar, 2022), the population is able to have 18.77 g of egg per person per day and which is averagely to 2.4 g of protein per person per day.

Table 4. Accuracy for Forecasting Results of Layer Egg Production (2000-2025)

Egg Production at	Forecasting Method	Validation Analysis		
		MAD	MSE	MAPE
Ft-3MA	Moving Average	153,176.881	84,627,810,766	16.23
Ft-3MWA	Weighted Moving Average	111,972.084	47,165,471,470	15.32
Ft-2MA	Moving Average	129,764.350	65,971,644,486	39.58
Ft-2WMA	Weighted Moving Average	129,764.350	63,222,825,965	39.58

Table 5. Geometric Method to Forecast Population of West Java for 2021-2025

Year	Population	Increase in population	% Increase in population	Projected Year	Population
2016	47,379,389			2021	50,310,377
2017	48,037,827	658,438	1.39	2022	50,687,705
2018	48,683,861	646,034	1.34	2023	51,067,863
2019	49,316,712	632,851	1.30	2024	51,450,872
2020	49,935,858	619,146	1.26	2025	51,836,753

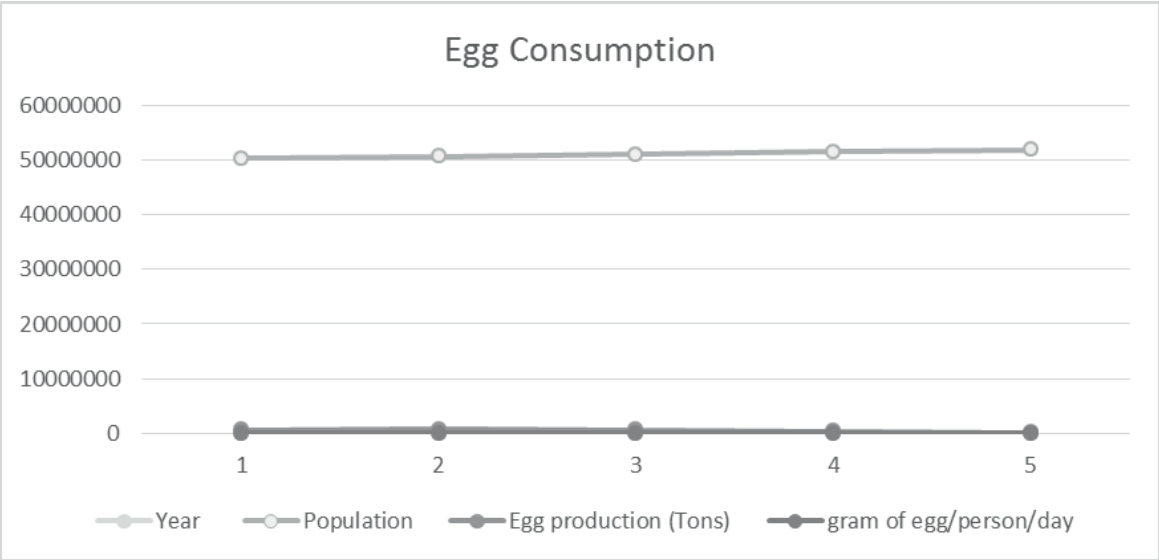


Figure 3. Projected Egg Consumption in West Java 2021-2025

According to Nutrition Adequacy figures of AKG of Indonesian people, the average protein intake should be 56.7 g of protein per person per day. In this case, the egg consumption contributes 4.2% to the protein consumed per person per day for the population in West Java (Headey, Hirvonen, 2018).

Based on the Figure 4, there is an increase in the population of West Java from 2021 to 2025 with an average increase of 1.3%. This study is further supported by a previous study conducted by Suryana and Sukandar about the forecasting of production and requirements of broiler chicken eggs for the consumption of animal protein in Aceh province which suggested that though nationally the production of broiler chicken eggs increased, the broiler chicken eggs produced in Aceh was still low to meet the needs of the Aceh people (Suryana & Sukandar, 2022 ; Sukandar, 2022). The production and consumption of eggs can be influenced by the various actors in the supply chain such as the government, breeding industry, breeders’ farmers groups as discussed in the study by Saptana in the Management of *Ayam Kampung* egg supply chain (Saptana & Sartika, 2014).

Furthermore, a study by Kristiansen about the Entry barriers in rural business, the case of egg production in Eastern Indonesia, suggested that entry barriers like capital costs, technological development, limited knowledge, and market dominance by powerful business groups limited

entry into the egg production business. Another study by Sukandar in the Discriminant Analysis to Determine the Poor Line Indicator reported that a farmer’s poverty line gold standard is 2.2g of gold/per capita/per month and the frequency of egg consumption used.

Policy guidelines must be developed to meet food security and sovereignty and engage all the stakeholders. The six pillars of food security, including availability, access, utilization, stability, sustainability, and system agencies, should be considered. The government can endorse agricultural policies that can promote and support egg production. This may include giving subsidies, grants and loans, modern equipment, and adopting advanced technologies. In addition, it can assist the farmers in constructing or expanding poultry farms. Research and development in the poultry sector can lead to innovations that can enhance egg production, such as new breeds with higher egg-laying capacities (Yaman et al., 2020), improve feed formulations and develop disease prevention and control strategies (Akintunde et al., 2015).

The concerned institutions can also organise effective training and education (Palada, 2020) to provide technical assistance to farmers, especially the small-scale producers about best practises for increased egg production and disease management in the poultry farm. The government can invest in an improved infrastructure such as upgrading hatcheries, feed mills, transportation, and storage

facilities to ensure the eggs reach the market in good condition. The marketing and distribution of layer eggs can be improved by establishing market linkages between producers and consumers through cooperative networks, quality control standards and consumer protection. The authorities can also implement effective disease control measures such as surveillance systems to monitor and control diseases that affect egg production, vaccination programs, biosecurity protocols and disease outbreak response.

CONCLUSION

Through the time-series data approach to Secondary Data Analysis, West Java is projected to have an average increment of 51,070,714 in its population by 2025 according to the Geometric method used. However, the Layer Egg Production that has been forecasted for 2023-2025 using MA and WMA in this province, is low to match the population demand to meet its protein contribution towards the nutrition adequacy as needed from animal food sources to fight against stunting. The population can have 18.77 g of egg per person per day and which is averagely to 2.4 g of protein per person per day which is 4.2% of protein contributed from egg. There are factors that are contributing towards this trend which may include income, population demand, cost of production and the availability of other protein sources, including plant proteins like *Tempe* and Tofu. This research can be further used to guide the stakeholders and policymakers to formulate policies and guidelines that enhance and facilitate increased production in the livestock sector for animal source foods, particularly layer egg production. This is crucial as it can uplift the provision of adequate, equitable and sustainable food which meets the nutritional requirements of the population at an affordable cost.

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II. FORMAT MANUSCRIPT WRITING

Manuscript should be typed using MS Word program, 1.5 space on A4 paper size with the left should be 4 cm length, while right, top, and bottom margin should be 3 cm length. The font used should be Times New Roman, sizing 16 pt for the title, 10 pt for author's name, 9 pt for author's identity, 11 pt for abstract and main content, and 9 pt for tables and figures. Specifically, for tables, single spaced should be used. The contents of the paper are made in two columns. The length of article should not below 10 pages and should not exceed 15 pages, send both in print-out and softcopy. Authors should also follow the manuscript preparation guidelines.

III. WRITING SYSTEM

Title in English

Author's Name (without degree)

Affiliation, City

E-mail:

Abstract in English (include keywords)

Introduction

Methods

Results and Discussion

Conclusion and Suggestion

References

Annotation

a. Title and Author's Identity

Title is written as clear, concise, informative, and understandable as possible. The maximum length of the title consists of 20 words. The author's name and identity (affiliation, city), are included

below the title. The name of corresponding author(s) is annotated by marking with numbers and (*) superscript. For undergraduate thesis, the name of thesis advisor is written as second author. **For undergraduate thesis, the name of thesis advisor is written as second author.**

b. Abstract and Keywords

Abstracts are written in English in Bahasa Indonesia and in Times New Roman 11 pt, single-spaced. Abstracts consist of no more than 250 words length, written in 1 (one) paragraph. Keywords are written below the abstract, consist of 3-5 specific words that are consistently used in the manuscript. Avoid using quotations and the use of abbreviations in writing abstracts.

Abstracts contain brief information regarding the background of the study, objectives of the study, brief summary of the methods (research design, subject selection, methods of data analysis), results, and discussion (use the most specific data in answering the objectives of the study, along with the signification results of statistical test, if any), conclusion as well as the significance/urgency of obtained conclusion..

c. Manuscript

The core section of a manuscript consists of subtitles: introduction, methods, results and discussions, and conclusion and suggestions.

Introduction

The introduction part comprises the background of the study, research intention, research questions, previous studies and the objectives of the study.

Methods

The methods section consists of the steps completed by the author in doing the research, elaborated completely, yet concisely, begins from research design (including the sampling methods, if any), samples, materials, & tools used, working methods, techniques of data collection, and data analysis.

Methods also include agreement from ethical commission (research involving human subject and/or animal experience).

Results and Discussions

Results of the study provide clear and concise results that are in line with the objectives of the study. The results can be complemented with tables and figures to help explaining the results.

- Number and title of a table are placed above the table and are written in bold. Table numbering is done in sequence. The lines used in table are only in table head and bottom (without column lines) with single space. Further explanations regarding the data on the table, the explanation can be written below the table.
- Number and label of figures are placed below figures and are written in bold. Figures numbering is done in sequence.
- Sources of reference are placed below tables/figures for tables and figures cited from other references (other than research results).

Discussions explains research results, concisely, and clearly. Using relevant arguments to the research topic and answering the research questions. Employ references (other research results or theories) to support the explanation of research. If there is abbreviation, use the standardized

abbreviations. The use of abbreviations must be preceded by the extensions first. Foreign terms are written in *Italics*. Numbers written in the beginning of a sentence are written in a word.

Conclusion

Conclusion elucidates important matters discussed in the result and analysis briefly, concisely, clearly, and answers research questions. Conclusion can be completed with suggestions (if necessary).

Acknowledgement (if necessary)

Acknowledgement given to person/institution who have important roles in conducting a research (for example, funders) and/or writing scientific manuscripts and includes explanations whether the research is part of a series of research in thesis/dissertation.

References

Writing references refers to the APA Referencing Guide 6th edition. [*Publication Manual of the American Psychological Association*. (6th ed.). (2010). Washington, D.C.: American Psychological Association]. References are arranged systematically and sorted alphabetically according to author's name. Generally, writing references is as follows:

Author, A.A., Author, B.B, & Author, C.C. (year of publication). *Title of publication: sub title*. (Edition [if not the first edition]). City of publication: Publisher.

A minimum of 80% of the literature used comes from 'up to date' sources (published no more than 10 years before scientific papers submitted to MGI). Unpublished sources, such as manuscripts or personal communication cannot be used as references for the writing.

EXAMPLES OF CITATION IN MANUSCRIPT

a. 1 author

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b. 2 author

Smith dan Jones (2017) or (Smith and Jones, 2017)

c. 3 or more authors

Smith, et al (2017) or (Smith et al., 2017)

EXAMPLES OF REFERENCES WRITING

a. References from books

- Contento, I. R. (2011). Nutrition education (2nd ed.). Sudbury, Massachusetts: Jones and Bartlett Publishers.
- Mahan, L. K., & Raymond, J. L. (2017). *Krause's food & the nutrition care process*. Canada: Elsevier Health Sciences.

b. Books or reports composed by organizations, associations, or government agencies

Kementerian Kesehatan. (2013). *Hasil Riset Kesehatan Dasar 2013*. Jakarta: Badan Penelitian dan Pengembangan Kesehatan, Kementerian Kesehatan RI.

c. Book chapters on a book that has editors

Brown, J.E. (2011). Nutrition through the life cycle (4th Ed.). Janet Sugarman Isaacs, *Infant Nutrition* (pp. 223–225). Belmont, CA, USA: Wadsworth.

d. **Conference manuscript – online**

Bochner, S. (1996). Mentoring in higher education: Issues to be addressed in developing a mentoring program. Paper presented at the Australian Association for Research in Education Conference, Singapore. Retrieved from <http://www.aare.edu.au/96pap/bochs96018.txt>

e. **Manuscripts from a journal**

El-Gilany, A. H., & Elkhawaga, G. (2012). Socioeconomic determinants of eating pattern of adolescent students in Mansoura, Egypt. *The Pan African Medical Journal*, 13, 22. <https://doi.org/10.4314/pamj.v13i1>.

McDonald, C. M., McLean, J., Kroeun, H., Talukder, A., Lynd, L. D., & Green, T. J. (2015). Correlates of household food insecurity and low dietary diversity in rural Cambodia. *Asia Pacific Journal of Clinical Nutrition*, 24(4), 720–730. <https://doi.org/10.6133/apjcn.2015.24.4.14>

Diana, R., Sumarmi, S., Nindya, T. S., Rifqi, M. A., Widya, S., & Rhitmayanti, E. (2017). *Household Income and Unbalanced Diet Among Urban Adolescent Girls. Proceedings of the 4th Annual Meeting of the Indonesian Health Economics Association (INAHEA 2017)*.

f. **Thesis/Dissertation – printed version**

Hilgendorf, M. (2018). *Assessing malnutrition in liver disease patients being evaluated for transplant using the nutrition focused physical exam* (Unpublished master's thesis). University of Kentucky, Lexington, Kentucky.

Diana, R. (2014). *Pengaruh pemanfaatan pekarangan dan penyuluhan terhadap konsumsi sayur dan asupan gizi rumah tangga dan balita*. Institut Pertanian Bogor.

g. **Thesis/Dissertation – web version**

Hilgendorf, M. (2018). *Assessing malnutrition in liver disease patients being evaluated for transplant using the nutrition focused physical exam* (Master's thesis, University of Kentucky, Lexington, Kentucky). Retrieved from https://uknowledge.uky.edu/cgi/viewcontent.cgi?article=1065&context=foodsci_etds

h. **Web page (if referenced are a few pages on the same web page, use the homepage page)**

SStatistic Bureau of East Java. (2018). Number and Percentage of Poor, P1, P2 and Poverty Line By Regency / Municipality, in 2017. Retrieved November 22, 2018, from <https://jatim.bps.go.id/statictable/2018/01/15/733/jumlah-dan-persentase-penduduk-miskin-p1-p2-dan-garis-kemiskinan-menurut-kabupaten-kota-tahun-2017.html>

Example of tables:

Table 1. Characteristics of Patients in Malnutrition and Non-Malnutrition Groups

Karakteristik	Malnutrition (n=70)		Non-Malnutrition (n=233)		Total (n=303)	X ²	p value
	n	%	n	%			
Sex							
Male	38	54,3	117	52,5	155	0,070	0,790
Female	32	45,7	106	47,5	138		
Age							
<55 years old	48	68,6	151	67,7	199	0,890	0,180
≥55 years old	22	31,4	72	32,3	94		
Education							
Low	24	34,3	51	22,9	75	10,153	0,063
Middle	33	47,1	151	67,7	184		
High	13	18,6	21	9,4	33		

Table 2. Average of Nutrition Intake in Malnutrition and Non-Malnutrition Groups

Nutrition Intake	Malnutrition (Mean ± SD)	Non-Malnutrition (Mean ± SD)	t	p value
Calories	1328,1± 215,3	1482,9± 327,4	2,04	0,032
Protein	43,2±13,1	48,7±17,3	2,47	0,010

Example of a figure:

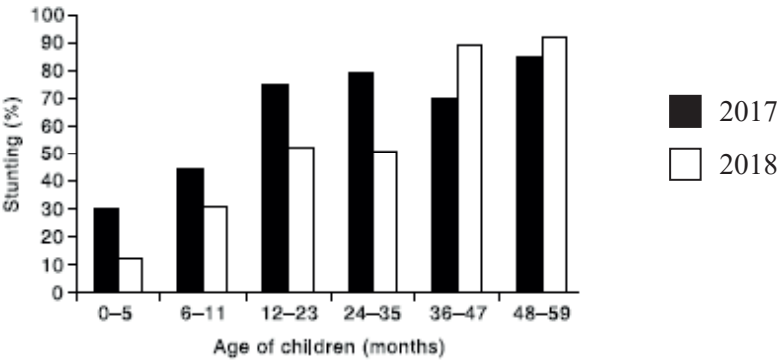


Figure 1. Changes in Stunting Prevalence (%) in Toddlers in Kalimantan

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