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Media Gizi Indonesia (MGI) has been published since 2004 is a scientific journal that provides articles regarding the results of research and the development of nutrition including community nutrition, clinical nutrition, institutional nutrition, food service management, food technology, current issues on food and nutrition. This journal is published once every 3 months: January, May, and September.

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INTRODUCTION TO THE EDITOR

Media Gizi Indonesia (MGI) is a scientific journal published regularly every 4 months that provides articles regarding the research and the development of nutrition knowledge including community nutrition, clinical nutrition, institutional nutrition, food service management, food technology, and current issues on food and nutrition. Media Gizi Indonesia tries to always present a variety of scientific articles in the scope of Nutrition and Health. This volume provides original research in the field of nutrition. The theme for original research focus on child nutrition and adolescent health. To date, child and adolescent nutrition has become more attention because a good nutritional status in that period will manifest a better quality of life during adults. Moreover, stunting reduction become the emerging issue. For that, this current edition of MGI presents several best research related to child and adolescent nutrition in relation to anemia, toddler development, and dietary intervention. Besides presenting studies related to child and adolescent nutrition, the current edition of MGI also shows research in food product development to provide better food product in order to alleviate nutritional problems. Futhermore, original research on food service management among athletes and nursing homes complements this volume.

We do hope MGI scientific journals can leverage the development of a writing culture and communicative scientific studies as well as attract readers and writers to participate in MGI for future issues. Media Gizi Indonesia will maintain its role in providing current, relevant, and topical issues in food and nutrition. Hopefully, the works displayed by MGI can provide benefits and enrich the readers' knowledge.

Editorial Team

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FISH-BASED COMPLEMENTARY FEEDING PRACTICES INCREASING MACRO AND MICRO NUTRITION INTAKE AND HEMOGLOBIN LEVELS IN ANEMIA TODDLERS

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ABSTRACT

Iron deficiency anemia in toddlers can be prevented by introducing complementary foods alongside breastfeeding. Fish, rich in protein and iron, offers a valuable nutritional source for young children, but proper processing is crucial. This research investigated the impact of fish-based complementary food processing practices (known as MP-ASI in Indonesia) on the nutritional intake and hemoglobin levels of children under five in Tiro District, Pidie Regency. Using a quasi-experimental design, this study involved 40 toddlers from the stunting-prone area of Tiro District, Pidie Regency, along with their mothers as respondents. The toddlers, aged 12-36 months without severe illnesses, were included in the study. The intervention comprised educating and practicing complementary food processing. Before-and-after intervention comparisons showed significant improvements in energy, carbohydrate, protein, zinc, and iron intake levels. Additionally, there was a notable increase in the average hemoglobin (Hb) level, rising by 1.06 g/dl after one month of fish-based complementary food processing intervention. In summary, this intervention effectively enhanced the nutritional quality of children's intake and increased their hemoglobin levels, contributing to the prevention of iron deficiency anemia in toddlers.

Keywords: anemia, nutritional intake, hemoglobin, fish consumption, education

INTRODUCTION

One of the priorities of the Program Indonesia Sehat through a Family Approach is reducing the prevalence of stunting. Stunting (low height for age) affects about a quarter of children aged <5 years worldwide (Gharpure et al., 2021). The problem of stunting in toddlers is the main nutritional problem faced in Indonesia.

According to SSGI 2022, Indonesia has a stunting prevalence of 21.6%. This figure is still above the threshold set by WHO, which is 20%. Based on national data, Aceh Province is ranked 5th in the highest prevalence of toddlers after East Nusa Tenggara Province and West Sulawesi Province. The success that the Aceh Government has achieved in reducing the stunting rate from 41.5% in 2013 to 37.3% in 2018 to 31.2% in 2022 (Kemenkes RI, 2014, 2018, 2022).

Stunting is a condition where children under five have a low height for their age. Stunted children experience malnutrition, which affects the maturity of nerve cells. Stunted children experience abnormal developmental delays such as slow motor movements, reduced intelligence, and slow social responses (Rosyidah et al., 2021). Several factors influence stunting, namely the age of the child, family size, number of children under five years in the household, wealth status, source of drinking water, eating habits, and food insecurity (Mengesha et al., 2021).

Previous studies stated that eating habits are one of the factors related to the incidence of stunting (Mulyaningsih et al., 2021). Eating habits affect the nutritional intake of stunted children. Hendraswari et al., pada tahun 2021 found that the intake of macronutrients such as energy and

protein intake is the main factor influencing the incidence of stunting. In addition, low iron is also a factor associated with stunting. Stunted toddlers generally have lower hemoglobin levels than toddlers who are not stunted (Flora et al., 2019; Losong & Adriani, 2017).

Nutrient intake for stunted toddlers can be done by providing additional food as a complementary food for breast milk. Providing additional food can increase nutritional intake, especially increasing hemoglobin levels (F. Wang et al., 2017; J. Wang et al., 2017). One additional food source of iron is derived from animal food sources (H.J. et al., 2011). Fish is a source of animal food with good nutritional content, especially a source of protein and iron (Safitri & Puspita, 2018; Syahril et al., 2016). Fish can also be used as a variety of innovative products such as biscuits, food bars, and cookies to address the problem of malnutrition, especially stunting, which can improve malnutrition status in children under five (Darawati et al., 2021; Susyani et al., 2022; Yuliana et al., 2019).

Based on this, the authors conducted this study to analyze the effect of fish-based complementary breastfeeding (in Indonesia: MP-ASI) processing interventions on the intake of macronutrients (Carbohydrate, protein, fat) and micronutrients (Vitamin A, C, calcium, zinc, and Fe) also Hemoglobin of toddlers. This activity was carried out in the stunting locus area designated as a sub-district with a high category of nutritional problems in Aceh, namely Tiro District, Pidie Regency.

METHOD

This type of research used a Quasi-Experimental one-group pretest-posttest design, where the research design was to compare before and after the intervention was given. This research was conducted in the Stunting Locus area, Tiro District, Pidie Regency, in July-August 2022.

The initial stage of this research was sampling, such as selecting villages, posyandu (an integrated health center), and households. The research subjects consisted of toddlers aged 12-36 months in the stunting locus area, Tiro District, Pidie Regency. There were 5 locus of stunting villages in the working area of the Tiro Health Center, namely

Pulo Masjid Village, Peuneudok Village, Pulo Siblah Village, Panton Beunot Village, and Panah Village. Sample calculations in this study using the Lemeshow formula obtained a minimum number of 36 people. Furthermore, respondents were taken by consecutive sampling by taking toddlers who met the inclusion criteria and obtained a total of 40 samples. Subject/sample inclusion criteria were toddlers aged 12-36 months, not currently seriously ill, their mothers, or respondents willing to participate in research activities up to signing informed consent.

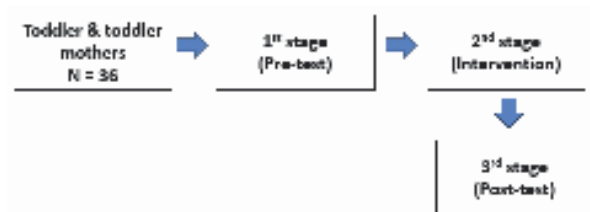


Figure 1. Research Stages

This research consisted of several stages: pretest/baseline, intervention I and II, and posttest (Figure 1).

1. Stage I, namely baseline data or pretest. At this stage, baseline data were collected, including data on household socioeconomic characteristics, subject characteristic data, macronutrient intake data (carbohydrates, protein, fat), and micro (Vitamins A, C, calcium, zinc, and iron) from subjects taken through 24-hour food recall method for toddlers. Meanwhile, data on blood hemoglobin levels were known in the fingertip capillaries using the digital easy touch method.
2. Stage II, the intervention was carried out 2 times during 1 month, 2 times 45 minutes each. The interventions carried out were in the form of nutritional counseling and behavior change practices in the form of processing fish-based complementary breastfeeding with demonstrations of processing fish-based complementary breastfeeding under the rules or principles of processing complementary breastfeeding for toddlers (guide in the recipe book). At the same time, the practice of processing fish-based complementary breastfeeding which was practice, was The practice of processing fish was carried out following the recipes contained in the recipe pocketbook. Nutrition education intervention

steps for respondents and posyandu cadres are as follows:

- a. Team opening
 - b. Administration of questionnaires before intervention
 - c. Collecting completed questionnaires
 - d. Explanation of material by the research team or resource persons, including 1) IYCF (Toddler Feeding); 2) problems of stunting and anemia; 3) the importance of fish in the growth and development of toddlers and fish-based complementary breastfeeding processing. Explanation of material using lecture and discussion methods using infocus tools, leaflets, and posters.
 - e. The team's practice of processing fish-based complementary breastfeeding included processing fish-based complementary breastfeeding menus high in nutrients to support growth and increase blood hemoglobin levels to prevent stunting and anemia. All participants would be given a fish-based complementary breastfeeding processing recipe book and leaf leads related to anemia in children under five.
3. The next stage was the posttest. The posttest stage consisted of collecting data on macronutrient intake (carbohydrates, protein, fat) and micronutrient intake (Vitamins A, C, calcium, zinc, and iron), blood hemoglobin levels in fingertip capillaries using the digital easy touch method.

The nutrient intake data collected was first converted to weight in grams, then the nutritional content was calculated, such as energy (kcal), protein (grams), fat (grams), calcium (grams), iron (mg), vitamin C (mg), and vitamin A (μg) using the Nutri-Survei 2007 program software. Then, it was compared with the nutritional adequacy figures for each toddler.

Microsoft Excel 2007 and SPSS 16.0 for Windows were used to analyze data descriptively and inferentially. Before testing the data in SPSS, the data normality test was first performed using the Kolmogorov-Smirnov test. The test for differences in normally distributed numerical data uses Paired T Test analysis to analyze the differences before and after the intervention of fish-based complementary breastfeeding processing practices on the adequacy of macro and micro-nutrients and hemoglobin levels of children under

five. Commission on Research Ethics Involving Human Subjects Number: LB.01.03/6/5621/2022.

RESULTS AND DISCUSSION

The sample characteristics in this study consisted of age, gender of the toddler, birth weight and height, and health history (Table 1). The sample in this study were toddlers aged 12 months to 36 months who were selected to meet the criteria, namely as many as 40 people. Most of the toddlers are in the age range of 12-24 months, namely 80.0%, male (60.0%), do not have LBW (Low Birth Weight) status (92.5%) and with birth length >45 cm (57.5%).

Children with birth weight lower than 2,500 g had a 5.9 times the risk of becoming stunted compared to children born weighing more than 2,500 g ($p < 0.05$; 95% CI: 0.93–37.8). Furthermore, children with birth length below 48 cm (short) had a 15.0 times higher risk of experiencing stunting ($p < 0.05$; 95% CI: 2.58–87.9) compared to children born with body length over 48 cm (Lukman et al., 2021). The child's birth weight and birth length are greatly influenced by the nutritional status and health of the mother before and during pregnancy, which affects the growth and development of the fetus during the neonatal period. Birth weight and length are closely related to the child's growth after birth. It was further explained that newborns with low birth weight have a higher risk of death in the first 28 days of life. Surviving people are more likely to suffer from stunted growth and lower IQs (Barker, 2007).

Table 1. Distribution of Toddler Characteristics

Toddler Characteristics	Number of toddlers	%
Age		
12-24 months	32	80.0
25 -36 months	8	20.0
Gender		
Male	24	60.0
Female	16	40.0
LBW status		
LBW (≤ 2500 g)	3	7.5
Not LBW (> 2500 g)	37	92.5
Birth length		
≤ 45 cm	17	42.5
> 45 cm	23	57.5

Intake of essential nutrients is related to the need for adequate health. The intake needs for several nutrients that must be met come from the food consumed. Nutritional adequacy is a comparison between nutritional needs and the intake of a particular individual or population, expressed as a percentage of recommended nutritional adequacy (Damara & Muniroh, 2021; Yuniar & Mahmudiono, 2022).

The sample nutrient intake data in this study were compared with the Nutrition Adequacy Rate based on the age of each toddler. The results obtained can be seen in Table 2.

Table 2. Average Adequacy Level of Energy and Other Nutrients Samples Before and After Intervention

Adequacy level	Pretest		Posttest		p-value
	Mean	SD	Mean	SD	
Energy (%)	43.3	18.75	60.9	14.2	0.00*
Carbs (%)	31.4	14.4	46.5	17.5	0.00*
Protein (%)	136.6	54.30	162.3	43.9	0.02*
Fat (%)	55.3	32.8	56.0	32.6	0.89
Vit A (%) Vit	149.6	247.6	113.4	182.8	0.36
C (%)	16.9	24.8	25.3	24.8	0.15
Calcium (%)	38.0	34.9	48.20	48.2	0.06
Zink (%)	94.6	54.4	122.0	50.9	0.00*
Fe (%)	52.1	27.3	66.8	32.5	0.00*

Table 2 shows the level of nutritional adequacy of the toddler samples in the study, most of whom were deficient or lacking, below 70% compared to the Nutrition Adequacy Rate. Only the average protein, vitamin A, and zinc values showed good results. The Paired T-test tests showed significant differences in the mean values before and after the intervention on the level of adequacy of energy, carbohydrates, protein, zinc, and iron. While the rest are not significantly different.

Fish is a source of animal food which is rich in nutrients. As a highly nutritious food, fish consumption is highly recommended for children for normal growth and development (Maulu et al., 2021). Fish has a high protein content with complete amino acids, so it can be developed as a good additional food for toddlers (Kadir, 2021). The high nutritional content in fish is

protein, calcium, oleic, palmitic, linoleic, and stearic acids. The highest amino acids are aspartic acid, glutamic acid, arginine lysine, and leucine (Nuryanto et al., 2022). Besides that, fish is also rich in micronutrients such as vitamins and minerals. Complementary foods substituted with fish have higher vitamin A and zinc content than complementary foods not mixed with fish (Hope et al., 2021).

Foods of animal origin derived from water, such as fish, can provide important nutrients and bioactive factors for human health. Animal food sources in the form of fish contain many nutrients such as docosahexaenoic acid [DHA], choline, vitamin B12, iron, and zinc. (Iannotti et al., 2022). Previous studies have shown that there is an increased effect of giving fish to anemic toddlers with a frequency of consumption of 7 times a week can improve the anemia status of children with indicators of increased blood profile in the form of ferritin, serum transferrin, and Hb. The existence of nutrition education interventions and fish-based complementary breastfeeding processing practices in this study can encourage mothers of toddlers to increase giving fish to their children. Intervention in giving fish to children who are anemic affects higher Hb repair and lower serum transferrin (Werner et al., 2022).

Hemoglobin levels

Test toddlers' Hb levels were measured twice before and after providing educational interventions and fish-based complementary breastfeeding processing practices. In Table 3, it can be seen that most of the samples of toddlers had anemia status, both before (87.5%) and after the intervention (77.5%). However, several samples showed a slight decrease in anemia status after providing nutritional education interventions and fish-based complementary breastfeeding processing practices.

Table 3. Anemia Status and Average Hb Levels of Samples Before and After Intervention

Anemia Status	Pretest		Posttest	
	n	%	n	%
Anemia	35	87.5	31	77.5
Not Anemia	5	12.5	9	22.5

Micronutrient deficiencies and stunting are significant problems in most children aged 6-59 months. The average Hb level of the samples before and after the intervention was in the low or anemia category (Ernawati et al., 2021). The incidence of anemia in stunted children is largely due to low consumption of animal foods (Mohammed et al., 2019). The average Hb level before the intervention was 8.64 g/dL and increased to 9.70 g/dL after the intervention. There was an increase in the average Hb level after giving counseling interventions and fish-based MP-ASI processing practices for 1 month of 1.06 g/dL. Even though there was an increase in posttest Hb levels, the average Hb level was in the anemia category.

Table 5. Average Hb Levels of Samples Before and After Intervention

Hb Level	Mean	SD	Mean	SD	p-value
Hb level	8.64	2.40	9.70	1.51	0.00*

The results of the different tests in this study used the Paired T-test. Table 4 shows a significant difference in the mean values of pretest and posttest Hb levels ($p=0.000$; $p>0.05$) before and after receiving nutritional education interventions and fish-based complementary breastfeeding processing practices. Anemia and stunting are closely related to a synergistic improvement in child health (Gaston et al., 2022). Previous studies have shown that most stunted children have a significant relationship with high anemia status (Rahman et al., 2019). Anemia is a comorbid factor in stunting that negatively impacts later life (Orsango et al., 2021). High anemia status in stunting toddlers is associated with increased cognitive abilities and lower physical growth of children (Orsango et al., 2021; Tampy, 2020). Demographic, socioeconomic, and geographic characteristics are also important drivers of stunting and anemia in children under 5 years (Gaston et al., 2022).

Factors of nutritional status and health can cause anemia in children. Malnutrition may not be directly related to anemia, but it leads to certain changes in the body that make it susceptible to health hazards that can cause anemia. Children and women who suffer from malnutrition are more likely to have a weaker immune system, making

them susceptible to various diseases and health hazards such as parasitic infections or chronic inflammation. Conditions like this can reduce hemoglobin levels in the blood, which causes an increase in the prevalence of anemia (Lukman et al., 2021). Stunted children of anemic mothers are at high risk for anemia (Lukman et al., 2021). This is explained based on certain factors that influence anemia and stunting. For example, mothers and children can have a healthy diet and access to micronutrient food sources rich in iron. Apart from that, they share the same things in the environment, have access to the same health facilities, and tend to have the same genetic characteristics. To overcome the problem of anemia in children, it is vital to design programs that target mothers and children. Empowering women or mothers of toddlers is one way to reduce the high prevalence of anemia and malnutrition in toddlers (Lukman et al., 2021).

CONCLUSION

There were significant differences in the average energy, carbohydrate, protein, zinc and iron intake values before and after the intervention. Increased energy intake, carbohydrates, protein, zinc, and iron were obtained after administering the intervention. There was an increase in the average Hb level after giving counseling interventions and fish-based complementary breastfeeding processing practices for 1 month of 1.06 g/dL. Further studies are needed with larger subjects and an extended number of intervention days to see the impact on anemia status.

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NUTRITION EDUCATION AND ASSISTANCE BASED ON BEHAVIOR CHANGE IN ADOLESCENT GIRLS TO IMPROVE NUTRITIONAL STATUS AND HEMOGLOBIN LEVELS

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ABSTRACT

Inappropriate nutritional behavior is a common cause of nutritional issues among adolescents, often leading to conditions like underweight and anemia. This study aimed to assess the impact of nutrition education and behavioral change-based support on the nutritional status and hemoglobin levels of adolescent girls. The study included 75 adolescent girls aged 16-18 years from Singosari Village, Gresik District, chosen through purposive sampling. Over a 30-day period, intensive nutrition education and assistance were provided, utilizing various methods such as lectures, discussions, role play, and practical exercises. The results revealed significant improvements in various aspects. Participants exhibited increased nutrition knowledge, energy and protein intake, body weight, nutritional status, and hemoglobin levels after the intervention ($p < 0.05$). The rise in knowledge was linked to higher protein intake ($p=0.009$), while adequate protein intake was associated with weight gain ($p=0.025$). Weight gain, in turn, correlated significantly with improved nutritional status based on BMI/U ($p = 0.041$). Hemoglobin levels also significantly increased, shifting from 11.6 ± 0.2 mg/dl (low) to 13.4 ± 0.1 mg/dl (normal) after the intervention. This improvement in hemoglobin levels was linked to increased energy intake ($p = 0.012$) and a change in the practice of consuming iron supplementation, shifting from no usage to weekly consumption. In conclusion, nutrition education and behavioral change-based support can effectively enhance energy, protein, and iron intake, leading to improved nutritional status and hemoglobin levels among adolescent girls.

Keywords: adolescent, education, assistance, hemoglobin, nutrition

INTRODUCTION

The nutritional status of women before conception is one of the influential factors during pregnancy and the health of the baby to be born. Malnutrition in pregnant women can be the cause of babies born having low birth weight and malnutrition in toddlers (Rahma and Nuradhiani, 2019). Malnutrition in pregnant women can also increase the risk of maternal death (UNICEF, 2018). The nutritional status and health of pregnant women are determined since adolescence or during their childbearing age (WUS) (Waskara, 2021). Currently, adolescents in Indonesia face three nutritional problems at once, including macronutrient deficiency (short and thin), micronutrient deficiency (anemia), and

overweight (overweight and obesity) (Baroroh et al. 2022).

Basic Health Research Data (RISKESDAS) in 2018 showed that the prevalence of short and very short in adolescents aged 13-15 years touched 25.7% while adolescents aged 16-18 years were 26.9%. Similarly, the prevalence of underweight and very thin adolescents, recorded as many as 8.7% of adolescents aged 13-15 years and 8.1% aged 16-18 years have underweight and very thin nutritional status. In contrast, the prevalence of adolescents with overweight and obese nutritional status aged 13-15 years was 16.0% and 13.5% aged 16-18 years. In addition to macronutrient problems, the problem of micronutrient deficiency such as anemia in adolescents in Indonesia is also fairly

high. The prevalence of anemia in adolescents is 32% (Ministry of Health RI, 2018).

Adolescents are prone to nutritional problems. Biologically, adolescents need more intake for organ growth and maturation (Bahar et al., 2020). Especially adolescent girls who periodically experience menstruation, so they are at greater risk of anemia (Hidayati et al. 2019). UNICEF (2017) collected data and obtained results, there were changes in consumption patterns and physical activity in adolescents, including adolescents doing the wrong food restrictions because they want to maintain weight, liking contemporary foods that tend to be high in calories and fast food, and the tendency of adolescents to choose foods that are not diverse are factors that play a role in three nutritional problems in Indonesian adolescents. There are also other supporting factors are social and educational inequality, to mental health problems.

UNICEF Indonesia in collaboration with the Government of Indonesia has begun pioneering a youth nutrition program designed to address three adolescent nutrition problems. This program applies a life cycle framework that aims to break the inter-generational chain of malnutrition. The program consists of three types of interventions, there are iron supplementation tablet containing iron and folic acid on a weekly basis to prevent anemia, nutrition education and behavior change. Providing nutrition education or nutrition education is one of the right methods to share information for adolescents and is supported by intensive mentoring which is an effort to change adolescent behavior. Research by Walilulu et al (2018), revealed that nutrition education has an effect on increasing knowledge and efforts to prevent nutritional problems. This study aims to examine the effect of education and nutrition assistance based on behavior change in adolescent girls on improving nutritional status and hemoglobin levels.

METHOD

The research design is quasi-experimental with pre- and post-one group design. This study involved 75 adolescent girls selected by purposive sampling with inclusion criteria aged 16-18 years,

physically and mentally healthy, not on a certain diet / strict diet and came from Singosari village, Gresik. The research began from July to September 2022 in Singosari Village, SMK DharmaWanita and SMK Semen Gresik. Interventions in the form of providing TTD once a week, education and mentoring are carried out for 30 days on active days and holidays with monitoring and evaluation carried out once a week. The main points of nutrition education provided are about the control and prevention of anemia, healthy eating behavior for adolescents. The material was delivered by researchers using the method of lectures and discussions at school gradually once a week for one month (30 days) with details of 4 times the provision of educational materials as well as 4 times taking blood added tablets together in class. Nutrition assistance is focused on monitoring iron supplementation tablet consumption practices and changes in healthy food consumption patterns. Blood tablets are given once a week and taken together in class. The actual consumption of respondents before the intervention was collected using the 24-h-recall method, then monitoring of respondents' consumption patterns was carried out by recording food (food record) and sending photos of food consumed through Whatsapp groups then recorded by enumerators. The results of recording Data collected during the study included the level of knowledge assessed through pre-test and post-test. Pre-test assessment is carried out before education and assistance and post-test is carried out at the end after the last intervention. In addition, consumption patterns, nutritional status (BMI / U) and hemoglobin levels. The tools used include questionnaire questions, 24-h-recall form and estimated food record, weight scale, microtoa and Easy Touch GCHb 3 in 1 hemoglobin test kit to measure Hb levels. Measurement of Hb levels was carried out by the research team. The stages of data analysis collected include normality tests, paired t tests to test for differences between before TTD, education and assistance and after intervention. Next, test pearson correlation to test the relationship between variables. This research has passed the ethical feasibility test by the ethics commission of Universitas Muhammadiyah Gresik No. 124/KET/II.3.UMG/KEP/A/2022.

RESULTS AND DISCUSSION

Characteristics of Respondents

A total of 75 young women were selected as respondents in this study. By age, 46.6% of respondents were 16 years old, 36.7% were 17 years old and 16.7% were 18 years old. All respondents came from Singosari Village, which is one of the stunting loci in Gresik Regency.

Nutritional Knowledge

Adolescence is a transition period from childhood to adulthood which includes biological changes, psychological changes, and social changes (Wulandari, 2014). Some studies say that education or counseling is one effective way to introduce balanced nutrition consumption patterns for adolescent girls (Ningsih, 2018). The knowledge gained by young women through education will influence attitudes, which then determine their behavior (Najahah, 2018; Nugraha et al. 2021).

The level of nutritional knowledge in this study was measured through pre-test and post-test results. As many as 58.7% of respondents had a lack of knowledge before being educated. After intensive education and assistance, nutritional knowledge increased significantly until 100% of respondents had a good level of knowledge ($p = 0.0001$) (Figure 1).

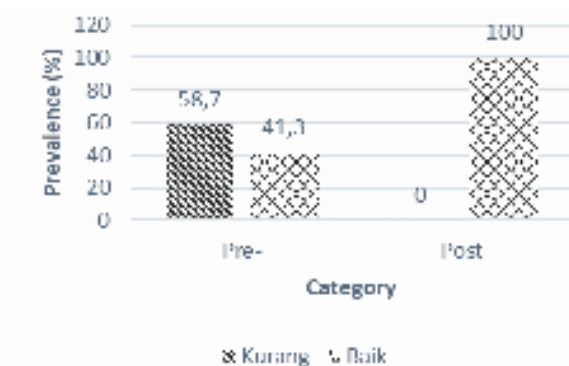


Figure 1. The Level of knowledge of respondents before and after the intervention

In this study, the intervention focused on intensive education and assistance related to behavior change. Education is aimed at increasing knowledge from adolescent girls related to healthy eating behavior and control and prevention of anemia for adolescents so that adolescents can live healthy, grow optimally and be free from anemia. Community-based nutrition education

methods have been widely applied with the aim of improving healthy living behaviors, preventing the emergence of diseases and improving the degree of health). Research by Jamaluddin et al (2020) shows that community-based nutrition education has positive results in the diversity of consumption patterns, hemoglobin levels, and children's attendance at school.

Knowledge is one of the domains that influence human behavior. Changes in behavior based on knowledge will be more consistent than behavior that is not based on knowledge (Notoadmojo, 2013). In practice, respondents with good knowledge do not always have good healthy living practices due to internal and external influences (Veriza & Riyadi, 2018). In this study, intensive assistance was applied as an effort to monitor good behavior that had been conveyed during education. Efforts made include respondents being given a food notebook, every meal students were asked to send photos of food consumed. If there is a discrepancy, respondents are motivated and counseled to meet needs according to their abilities.

Consumption Patterns

The results of recording energy intake before the intervention showed that as many as 66.7% of respondents had energy intake with the category of severe deficit, 9.3% moderate deficit, 8% mild deficit and 16% sufficient with the average energy intake of respondents as 1201 + 34.6 kcal. Not much different from energy intake, more than 50% of respondents protein and carbohydrate intake were categorized as weight deficit (Table 1). This can be the cause of growth and development of young women is not optimal.

Table 1. Classification of respondents' energy and nutrient intake before intervention

Category	Energy Intake (%)	Protein Intake (%)	Fat Intake (%)	Carb Intake (%)
Severely Deficit (<70%)	66,7	56	9,3	70,7
Moderate Deficit (70-79%)	9,3	22,7	4	13,3
Mild Deficit (80-89%)	8	13,3	6,7	8
Adequate (90-120%)	16	8	52	8
Overintake(>120%)	0	0	28	0

Adolescents need a greater intake of nutrients than during childhood. However, teenagers tend to have a diet that is not in accordance with needs. After being educated and mentored for one month, there was a significant increase in energy, protein, fat and carbohydrate intake (Table 2).

Table 2. Results of recording respondents' food intake (24-h recall and estimated food record)

Category	Pre-	Post-	Δ	P value
Energy intake (Kkal)	1201 ± 34,6	1334 ± 37,9	133 ± 15,2	0,0001*
Protein intake (g)	45,6 ± 0,7	50,2 ± 0,9	4,5 ± 0,7	0,0001*
Fat intake (g)	51,0 ± 1,2	55,0 ± 1,2	3,9 ± 0,8	0,0001*
Carb. intake (g)	164,8 ± 9,6	171,4 ± 5,4	6,5 ± 7,6	0,393

Keterangan : uji *t* berpasangan signifikan pada $\alpha < 0,05$

Young women's energy intake increased after education and mentoring by 133+ 152 kcal (Table 2). The increase in total energy cannot be separated from the increase in intake of energy-contributing nutrients, namely protein ($\Delta=4.5 + 0.7$ g), fat ($\Delta=3.9 + 0.8$ g) and carbohydrates ($\Delta=6.5 + 7.6$ g). The increase in protein intake occurred due to changes in the selection of vegetable and animal protein sources that previously rarely consumed vegetable protein to choose fried tempeh as a source of animal protein with a frequency of 4-6 times per week.

The selection of animal protein sources from respondents also changed, which previously on average liked sausages (ready-to-eat foods) consumed 4-6 times per week turned into various processed chicken. Based on data on the composition of Indonesian food, the protein content of sausages is lower than processed chicken. The protein content in 100 g of sausage is only 14.5 g while in fried chicken has a protein content of between 31-35 g per 100 g depending on the body part of the chicken (thigh = 32.1 g; wings = 35 g and breast = 34.2 g). The amount of protein content in processed chicken is twice as much compared to sausages. Consumption of protein that is less and not diverse will greatly impact the condition of growth and development of adolescents (Wulandari, 2014).

Fat intake in respondents also increased. This is related to an increase in protein intake, where the source of protein is also a source of fat. Ready-to-eat sausages sold in the market contain 27 g of fat per 100 g while chicken has various contents according to its part (thigh = 15.7 g; wings = 15.2 g and breast = 16.2 g). Although chicken seems to have less fat content than sausage, the most common chicken processing is fried (such as crispy chicken, chicken katsu, geprek chicken) so as to increase the amount of fat intake from the oil used. Based on the oil absorption table, the absorption of cooking chicken oil is 16%.

Teenagers are one of the groups that dominate the consumption of ready-to-eat foods. The high consumption of ready-to-consume food products is associated with practical products and delicious taste (Godatwar et al. 2015). The level of nutritional knowledge has an impact on a person's attitude and behavior in food selection and the formation of eating habits. Lack of nutritional knowledge about a good diet and lack of understanding of the role of nutrients from various types of food will cause problems not only in nutritional status and health, but also affect intelligence and productivity (Soekirman, 2011).

The nutritional status of adolescent girls is determined by BMI / U which begins with measuring the weight and height of respondents. The average body weight of respondents before the intervention was 53.4 + 2.1 kg. After following the intervention there was a significant weight gain to 54.5 + 2.02 kg ($p = 0.0001^*$) (Table 2). The height measurement result was 1.56 + 0.01 m and did not change between before and after the intervention.

Table 3. The results of anthropometric measurements

Variabel	Pre	Post	P value
BW (Kg)	53,4 ± 2,1	54,5 ± 2,02	0,0001*
Height (m)	1,56 ± 0,01	1,56 ± 0,01	1

Keterangan: uji *t* berpasangan signifikan pada $\alpha < 0,05$

The results of nutritional status classification based on BMI / U before the intervention showed that as many as 42.3% of respondents were underweight, 26.7% Normal, 24% overweight and 8% obese. After the intervention, the prevalence of underweight adolescent girls decreased to 34.7%

and those with normal nutritional status increased to 33.3% (Table 3).

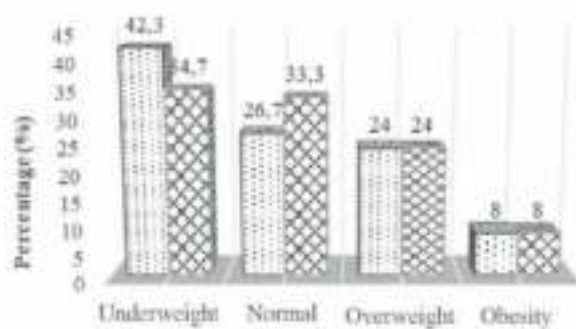


Figure 2. Classification of nutritional status of respondents before and after the intervention

Weight gain is associated with improved nutritional status based on BMI/U ($p = 0.04^*$). The diet of adolescent girls will determine the amount of nutrients needed for growth including weight gain (Mokoginta et al. 2016). Weight gain is also linear with increasing BMI values, so that improvements in nutritional status can be achieved.

The number of overweight and obese adolescent girls did not change between before and after the intervention. This is because research focuses more on improving the quality of consumption (sufficient quantities and diverse types) but does not increase physical activity. The message of the Nusantara Movement to Press Obesity Rates (GENTAS) from the Ministry of Health in an effort to reduce the prevalence of more nutrition is to regulate diet, one of which is the amount of food sources of protein equivalent to food sources of carbohydrates, increase consumption of fruits and vegetables besides that it also increases body movement (physical activity and physical exercise) (Ministry of Health RI, 2017).

In this study, there was an increase in the amount of protein intake as well as an increase in the frequency of fruit and vegetable consumption. Before the intervention, only 30% of respondents consumed fruit daily. After intensive education and assistance, the number of respondents who consumed fruit every day increased to 47% both directly consumed and consumed in the form of juice. As for vegetable consumption, as many as

23.7% of respondents consumed vegetables every day before the intervention and increased to 40%.

Recommendations for fruit and vegetable consumption in someone who experiences more nutrition are at least equivalent to protein and carbohydrates. Consumption of fruits and vegetables in greater quantities has a dual function, namely as a source of fiber and a source of vitamins and minerals. The fiber content in fruits and vegetables can provide satiety in a longer time and can help the body eliminate piles of food in the large intestine. Vitamins and minerals in vegetables and fruits are useful for maintaining health, especially counteracting free radicals due to excess fat accumulation in people who are obese (Ministry of Health RI, 2017).

Hemoglobin Level

The results of measuring respondents' hemoglobin levels before the intervention showed that as many as 62.7% of respondents had anemia with an average hemoglobin level of respondents 11.6 ± 0.2 g / dL. After education and assistance and regular consumption of blood-added tablets once a week, the average hemoglobin level increased significantly to 13.4 ± 0.1 g/dL ($p = 0.0001^*$). The Pearson correlation test showed that increased hemoglobin levels were significantly associated with increased energy intake ($p = 0.012^*$), and were associated with increased TTD consumption. After this research was completed, the task of monitoring TTD consumption was returned to the school.

Research Susanti et al. (2016) explained that iron supplementation on a weekly basis has the same effectiveness in increasing hemoglobin levels in adolescent girls with daily consumption of TTD during menstruation. Giving TTD to adolescents is recommended once a week with an excess level of adherence to higher supplement consumption.

CONCLUSION

Based on the results of the study above, providing education and nutritional assistance based on intensive behavior change can increase knowledge, increase energy, protein, fat and carbohydrates and regular consumption of TTD once a week. The improvement of knowledge

and daily consumption patterns and blood-added tablets is significantly related to improvements in nutritional status (BMI / U) and hemoglobin levels in adolescent girls.

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CAVA SMOOTHIE AS AN ADJUVANT IN IRON SUPPLEMENTATION CAN INCREASE HEMOGLOBIN LEVELS AND ERYTHROCYTE INDICES IN ANEMIC ADOLESCENT GIRLS

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ABSTRACT

Uncontrolled anemia leads to a decline in hemoglobin levels and erythrocyte blood indices, posing various health risks. To address this, a study aimed to assess the effectiveness of cava smoothies and iron supplementation in boosting hemoglobin levels and erythrocyte indices among anemic adolescent girls. The study involved 87 adolescent girls with moderate anemia, divided into three groups. The control group (C) received weekly iron tablets (60 mg), while two intervention groups (I1 and I2) were given the same iron tablets along with cava smoothies in increasing doses over 28 days. The results revealed significant improvements in hemoglobin levels. Group C showed an increase of 0.27 g/dL, while I1 and I2 demonstrated remarkable gains of 1.46 g/dL and 2.54 g/dL, respectively. Furthermore, erythrocyte indices (MCV, MCH, MCHC) exhibited positive changes. Group C saw increases of 0.35 fl, 0.34 pg, and 0.27 g/dL, I1 showed improvements of 0.69 fl, 0.61 pg, and 0.64 g/dL, and I2 experienced substantial enhancements of 1.97 fl, 1.61 pg, and 1.60 g/dL. In conclusion, the study confirmed that cava smoothies and iron supplementation effectively raise hemoglobin levels and improve erythrocyte indices in anemic adolescent girls, highlighting the potential benefits of this intervention for combating anemia.

Keywords: anemia, cava smoothie, hemoglobin, erythrocyte indices, iron supplementation

INTRODUCTION

Anemia is defined as a condition in which the hemoglobin levels are insufficient to meet the body's physiological needs (Miles & Richards, 2022). Anemia is associated with physical impairments, reduced quality of life, increased susceptibility to infections, elevated morbidity and mortality rates in women and children, decreased work productivity, cognitive impairments, and developmental disorders in preschool-age children and reproductive-aged women (Chaparro & Suchdev, 2019; Richards *et al.*, 2021). Individuals with anemia are more commonly found among adolescent girls, as adolescent girls are relatively ten times more likely to experience anemia compared to adolescent boys (Kemenkes RI, 2018). The prevalence of anemia in Southeast Asia ranges from 14.0% to 46.0%, while in Indonesia, it stands at 28.0%, making Indonesia the fifth-ranked country in Southeast Asia regarding anemia

prevalence (World Health Organization, 2016). Anemia due to iron deficiency, or Iron Deficiency Anemia (IDA), is an ongoing nutritional issue in Indonesia. Data from the Basic Health Research (Riskesdas) indicates that the prevalence of IDA among adolescents in Indonesia has increased from 37.0% in 2013 to 48.1% in 2018 (Martiasari *et al.*, 2022; Badan Penelitian dan Pengembangan Kesehatan, 2018).

The management of anemia in adolescent girls can be carried out through a government program known as the Iron Supplementation Tablets (IST) program (Yudina & Fayasari, 2020). Circular Letter from the Ministry of Health of the Republic of Indonesia Number HK.03.03/V/0595/2016 regarding the provision of IST to Adolescent Girls and Women of Reproductive Age stipulates that IST should be given at a rate of 1 tablet per week per year (≥ 52 tablets/year). The consumption rate of IST in Indonesia remains low,

with 98.6% of adolescent girls consuming fewer than 52 tablets per year and only 1.4% meeting the recommended intake (Nurcahyanti *et al.*, 2022). The reason adolescent girls do not consume iron-fortified instant drinks (IFID) is due to feelings of boredom or laziness, as well as the unpleasant taste and aroma of IFID. Some adolescent girls also experience side effects such as nausea and upper abdominal pain (Ningtyias *et al.*, 2020). Innovative supplementary beverages rich in iron and Vitamin C are required to increase hemoglobin levels and erythrocyte indices.

Several food items can be utilized to help increase hemoglobin levels and erythrocyte indices, such as *Cavendish bananas*, butter avocados, pure honey, and Sukkari dates. Bananas are a commonly found fruit in many countries, particularly in Asia (Garcı *et al.*, 2015). The iron and vitamin C content in *Cavendish bananas* is relatively high, with 2.6 mg and 55.1 mg per 100 grams, respectively, both of which can assist in enhancing hemoglobin levels and erythrocyte indices (Aryani *et al.*, 2022). *Cavendish bananas* contain 0.7 grams of protein, 0.1 grams of fat, and 19.8 grams of carbohydrates per 100 grams (Aryani *et al.*, 2022). Avocado fruit has the potential to aid in addressing anemia in adolescent girls, as 100 grams of avocado contains 0.6 mg of iron and 8.80 mg of vitamin C (Dreher & Davenport, 2013). Avocado also contains 0.4 g of protein, 2.0 g of fat, and 8.7 g of carbohydrates in every 100 grams (Viera *et al.*, 2022). Honey can increase hemoglobin levels and erythrocyte indices in the blood, as it contains 6.4 mg of iron and 10.7 mg of vitamin C every 100 grams (Islam *et al.*, 2017). Honey also contains 6.1 g of protein, 0.5 g of fat, and 55.3 g of carbohydrates per 100 grams (Mustafa *et al.*, 2023). Dates also possess the potential to assist in alleviating anemia in adolescent girls. This is caused by the fact that 100 grams of dates contain 10.4 milligrams of iron and 1.71 milligrams of vitamin C (Karajibani, 2019; Olabinjo *et al.*, 2022). Additionally, within the same 100-gram portion, dates provide 2.0 grams of protein, 3.6 grams of fat, and 82.6 grams of carbohydrates (Zar Pasha *et al.*, 2022).

The current popular beverage trend is the smoothie, which is primarily sold in fast-food

establishments (Šilha *et al.*, 2022). Smoothies are commonly consumed by children and adolescents (Fidler Mis *et al.*, 2017). Smoothies not only offer a wealth of nutritious and healthful ingredients but also stand out due to their distinctive and unique presentation, making them more appealing to consumers (Malau *et al.*, 2019). The preliminary examination conducted by the researcher determined that the composition of cava smoothie in 100 grams includes 4.1 grams of protein, 1.0 grams of fat, 30.6 grams of carbohydrates, 41.2 milligrams of vitamin C, and 12.5 milligrams of iron.

Many studies indicate that bananas, avocados, honey, and dates are local food sources with the potential to control anemia. The first study is being conducted by Rifiana & Hardiani (2021), which shows that the consumption of 100 grams of banana significantly increases hemoglobin levels over a 14-day period in anemic adolescent girls. Related research on the utilization of avocados is also being carried out by Feriyal (2017), showing that the consumption of 200 grams of avocado juice significantly increases hemoglobin levels over a 14-day period in anemic adolescent girls. Meanwhile, research on the potential of honey was conducted by Cholifah & Wulandari (2018), demonstrating that giving 3 tablespoons of honey per day for 14 days can significantly increase hemoglobin levels in anemic adolescent girls. Additionally, a study conducted by Susilawati (2022) indicates that the consumption of four dates over 7 days has a significant impact on increasing hemoglobin levels in anemic adolescent girls.

Although several researchers have conducted research on bananas, avocados, honey, and dates, a study on the combined effects of banana, avocado, honey, and dates in the form of a cava smoothie to improve hemoglobin levels and erythrocyte indices over a 28-day period, with the addition of iron supplementation, has never been undertaken. Therefore, this represents a novelty in research. Based on the description above, the researchers are interested in verifying whether a cava smoothie and iron supplementation can increase hemoglobin levels and erythrocyte indices in anemic adolescent girls.

METHOD

Data source and study design

This study was a quasi-experimental research with a pretest-posttest control group design. The study subjects consisted of adolescent girls with anemia, and the inclusion criteria were as follows: Adolescent girls with hemoglobin levels between 8.0-10.9 g/dL (experienced moderate anemia), aged 13-18 years, had regular menstruation occurring once a month (with cycles ranging from 21-35 days) for approximately 7 days, had a preference for bananas, avocados, honey, and dates, and did not suffer from chronic illnesses (such as intestinal parasites and malaria). Exclusion criteria included: Absence during data collection, illness during the study, and unwillingness to consume the cava smoothie. The research was conducted in five Islamic boarding schools in Metro City: Roudlotut Tholibin Islamic Boarding School (North Metro), Roudlatul Qur'an Islamic Boarding School (West Metro), Darul A'mal Islamic Boarding School (West Metro), Muhammadiyah At-Tanwir Islamic Boarding School (West Metro), and Daarul Ulya Islamic Boarding School (East Metro) in January-February 2023. The reason for selecting these five Islamic boarding schools as research locations was based on secondary data from the Metro City Health Office (year 2022), indicating that these boarding schools were in the top three areas with the highest prevalence of anemia in Metro City. From the population in these five boarding schools, a purposive sampling method was used to obtain a sample of 87 adolescent girls with moderate anemia.

The research subjects were then randomly divided into 3 groups, with each group consisting of a mix of individuals from various Islamic boarding schools. Each group comprised 29 adolescent girls, namely: Group C (Control Group), consisting of adolescent girls with anemia who were only given iron supplementation; Group I1 (Intervention Group 1), consisting of adolescent girls with anemia who were given iron supplementation (60 mg) and a cava smoothie at a dosage of 100 ml; and Group I2 (Intervention Group 2), consisting of adolescent girls with anemia who were given iron supplementation (60 mg) and a cava smoothie at a dosage of 200 ml. All adolescent girls with anemia

were provided with iron supplementation in the form of Blood-Boosting Tablets at a dosage of 60 mg once a week if they were not menstruating and 7 times a week if they were menstruating. Adolescent girls with anemia were also given Cava Smoothie in two dosages: 100 ml and 200 ml every 2 days. The compliance level of the respondents in consuming iron supplementation and Cava Smoothie was assessed through the completion of questionnaires/forms in which they recorded the timing of iron supplementation and Cava Smoothie consumption, accompanied by their signatures and the endorsement of the responsible individuals at the Islamic boarding schools.

Research tools and materials

The research tools used to prepare the cava smoothie included: a blender, plastic cups, disposable hand gloves, measuring spoons, a kitchen scale, a knife, standing pouches, a refrigerator, and an ice cooling box. The research tools used for blood sample collection included: disposable gloves, 3 ml injection syringes, 3 ml EDTA tubes, 70% alcohol swabs, adhesive bandages, and a tourniquet. Meanwhile, the instrument used for analyzing blood serum (hemoglobin levels and erythrocyte indices) was the Dirui BC 3600 Hematology Analyzer.

The ingredients used to make a cava smoothie include: ripe white *Cavendish bananas* and ripe buttery avocados, pure honey, and Sukkari dates. The bananas and avocados were sourced from plantations in Lampung Province. Ripe bananas were selected based on characteristics such as bright, evenly yellow skin extending from the middle to the tips, a rounded shape, and smooth and soft banana skin (picked at 3 months of fruit age). The ripe avocados were selected based on characteristics such as dark green to deep purple skin, a soft texture, easy separation of the fruit from the skin, and easy removal of the seed from the flesh (picked at 6-7 months of fruit age). Pure honey and Sukkari dates were obtained from the Tayyiba store in Surakarta City.

The materials for the examination of hemoglobin levels and erythrocyte indices include 3 ml of blood drawn from a vein, 70% alcohol, and EDTA. Reagents for the examination of

hemoglobin levels and erythrocyte indices consist of hematology reagents such as Lyse, Cleanser, Diluent, and roll paper.

Preparation of cava smoothie

The cava smoothie was made from the flesh of Cavendish bananas, buttery avocados, pure honey, and Sukkari dates. The process of making Cava Smoothie began with handwashing and cleaning of utensils and ingredients. Plastic gloves were then worn. Cavendish bananas and buttery avocados were peeled and sliced. Slices of Cavendish bananas (75 g) and buttery avocados (75 g) were placed into a standing pouch and stored in the freezer overnight at a temperature of -17°C. After one night, once the bananas and avocados had frozen, they were transferred into a blender container. Pure honey (25 ml) and Sukkari dates (25 g) were added to the blender. All the ingredients were blended until smooth and homogeneous. The smoothie was then poured into 100 ml and 200 ml plastic cups (according to the research dosage). To keep the cava smoothie cold, it was placed in an ice-cooling box. The cava smoothie was ready to be served to the research subjects.

The gives of iron supplements and cava smoothie.

Before being given iron supplementation and cava smoothie treatment, research subjects were instructed on the stages that would be conducted in the study and a commercial brand of deworming medication (albendazole). The deworming medication was given as a single dose of 400 mg and taken 21 days before the treatment began. Iron supplementation was achieved by providing commercial-brand iron supplement tablets (iron-fortified tablets) containing 60 mg of iron per tablet. Iron supplement tablets were given once a week to research subjects who were not menstruating, and for research subjects who were menstruating, they were given once a day for 7 consecutive days, and after menstruation, they were given once a week. Iron supplement tablets were provided to all groups, including the control group (C) and the intervention groups (I1 and I2). In addition to receiving iron supplementation, the intervention groups also received a cava

smoothie every 2 days. Group I1 received cava smoothie dose 1 (100 ml) every 2 days, while group I2 received dose 2 (200 ml) every 2 days. The treatment with iron supplements and a cava smoothie was carried out over a period of 28 days.

The examination of Hb levels and erythrocyte indices

The examination of Hb levels and erythrocyte indices to determine the effect of cava smoothie was conducted before and after the treatment, specifically 1 day before the treatment (H0) and after 28 days of treatment (H29). Blood collection and the examination of Hb levels and erythrocyte indices were performed at the Iringmulyo Public Health Center Laboratory, East Metro. Blood was drawn from the cubital vein using a 3 ml injection syringe, and then the blood was collected into a 3 ml EDTA tube (serum tube). Subsequently, the blood in the tube was diluted with Dirui BC 3600 reagent. The measurement of Hb levels and erythrocyte indices was performed using the impedance method, automatically calculating red blood cells (Isma et al., 2017). Hb levels are expressed in units of g/dL, while erythrocyte indices include MCV (mean corpuscular volume) expressed in units of fl, MCH (mean corpuscular hemoglobin) expressed in units of pg, and MCHC (mean corpuscular hemoglobin concentration) expressed in units of g/dL.

Data analysis

In this study, to determine the differences in Hb levels and erythrocyte indices before and after treatment within each group, data were analyzed using the Paired T-Test (for normally distributed data). However, for data with non-normal distribution, the Wilcoxon test was used for analysis. To determine the differences in the hemoglobin level and erythrocyte indices among the groups, the data were analyzed using the One-Way ANOVA test, followed by Post Hoc tests (for normally distributed data). However, for data with non-normal distribution, the Kruskal-Wallis test was used, followed by the Mann-Whitney test (Sumardiyono, 2020).

In this study, the significance level used is $\alpha=0.05$. The analysis results are considered

significant if the p-value is less than 0.05 ($p < 0.05$), and they are considered not significant if the p-value is greater than or equal to 0.05 ($p \geq 0.05$).

Ethical approval

All procedures conducted in this study have been approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Sebelas Maret Surakarta, with letter number 119/

UN27.06.11/KEP/EC/2022 dated September 26, 2022.

RESULTS AND DISCUSSION

Respondent Characteristics

Table 1 presents the characteristics of the respondents, including Age, Education, and Hb Levels.

Table 1. Subject Characteristics

Variable	Category	Group						p-value
		C		I1		I2		
		N	%	N	%	N	%	
Age	Early Adolescents (10-13 years)	1	3.4	3	10.3	3	10.3	0.399
	Middle Adolescents (14-17 years)	26	89.7	24	82.8	25	86.3	
	Late Adolescents (18-24 years)	2	6.9	2	6.9	1	3.4	
	Total	29	100.0	29	100.0	29	100.0	
Education	Junior High School (SMP/MTs)	12	41.4	21	72.4	17	58.6	0.057
	Senior High School (SMA/MA)	17	58.6	8	27.6	12	41.4	
	Total	29	100.0	29	100.0	29	100.0	
Hb Levels	Mild Anemia (11,0-11,9g/dL)	0	0.0	0	0.0	0	0.0	0.332
	Moderate Anemia (8,0-10,9g/dL)	29	9.5	29	10.3	29	10.2	
	Severe Anemia (<8,0g/dL)	0	0.0	0	0.0	0	0.0	
	Total	29	100.0	29	100.0	29	100.0	

Note: C = Control Group (given iron supplementation 60 mg/week); I1 = Intervention Group 1 (given iron supplementation 60 mg/week and cava smoothie 100 ml/every 2 days); I2 = Intervention Group 2 (given iron supplementation 60 mg/week and cava smoothie 200 ml/every 2 days).

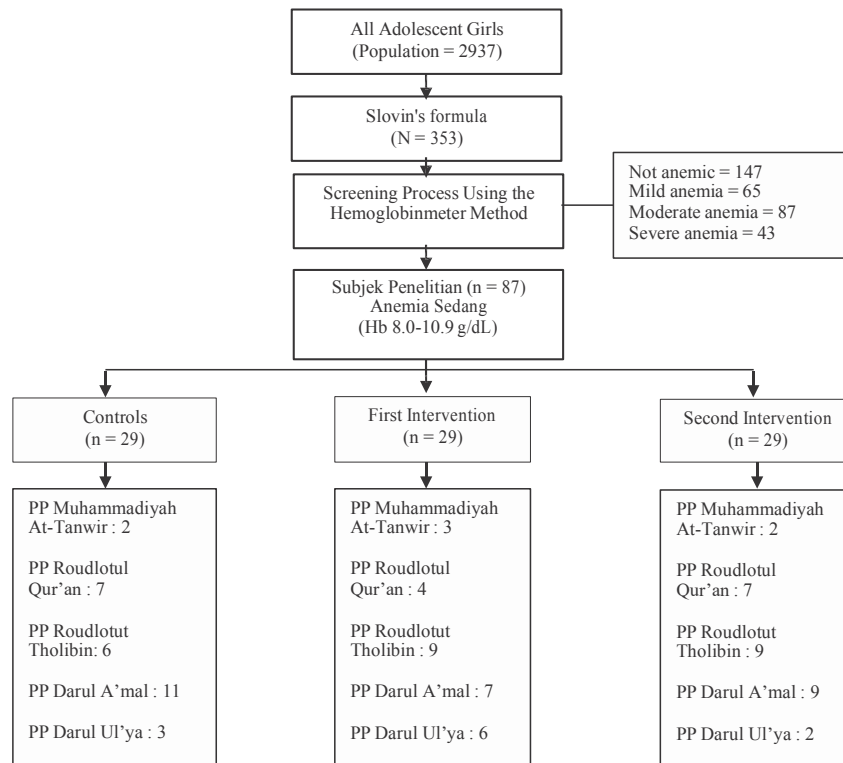


Figure 1. Research Subject Allocation Flowchart

Note: PP = Islamic Boarding School

Figure 1 illustrates that to divide the research subjects, it can begin by collecting all adolescent girls from 5 Islamic boarding schools in Metro City, totaling 2937 adolescent girls. Then, using Slovin’s formula, 353 adolescent girls were selected. From these 353 adolescent girls, a screening process was conducted using the hemoglobinometer method, resulting in 147 non-anemic adolescent girls, 65 with mild anemia, 87 with moderate anemia, and 43 with severe anemia.

The researchers selected adolescent girls with moderate anemia because the intervention used was appropriate for addressing it, which is the provision of iron supplementation and cava smoothie. The researchers did not choose adolescent girls with mild anemia because mild anemia can be addressed simply by consuming iron-rich foods, whereas severe anemia cannot be addressed with just iron supplementation or cava smoothie alone. In other words, severe anemia requires more serious treatment compared to mild and moderate anemia.

The 87 research subjects, who were adolescent girls with anemia, were divided into 3 groups randomly, with each group consisting of 29 adolescent girls with anemia. These 29 adolescent girls with anemia were a combination of individuals from 5 Islamic boarding schools in Metro City.

Table 1 shows that the total research subjects were 87 respondents, with the majority of adolescent girls falling into the mid-teenage age group (14-17 years old), totaling 72 individuals. The highest educational level among the adolescent girls was junior high school (SMP/MTs), with 50 individuals. All adolescent girls had hemoglobin (Hb) levels categorized as moderate anemia, ranging from 8.0-10.9 g/dL. Based on the results of statistical tests, it was found that there was no significant difference among the three groups in terms of age, education, and Hb levels, indicating homogeneity among the research subjects (p -value > 0.05).

Giving Cava Smoothie and Iron Supplementation Increases Hemoglobin Levels

Table 2. Mean Hemoglobin Levels on Day 0 and Day 28, and the Difference in Each Group

Group	Mean ± SD (g/dL)			Kruskal-Wallis Test
	C	I1	I2	
Day 0	10.14 ± 0.60	10.05 ± 0.68	9.90 ± 0.78	0.653
Day 28	10.41 ± 0.29	11.51 ± 0.84	12.44 ± 0.79	0.001*
▲ (g/dL)	0.27 ± 0.57 ^a	1.46 ± 0.96 ^b	2.54 ± 1.24 ^c	0.001*
Wilcoxon Test	0.140	0.001*	0.001*	

Note: C = Control Group (given iron supplementation 60 mg/week); I1 = Intervention Group 1 (given iron supplementation 60 mg/week and cava smoothie 100 ml/every 2 days); I2 = Intervention Group 2 (given iron supplementation 60 mg/week and cava smoothie 200 ml/every 2 days); a, b, c = Significantly different in Mann-Whitney Test; * = Significantly different (<0.05).

Figure 2. Bar Chart of Mean Hemoglobin Levels on Day 0 and Day 28 in Each Group

Legend: C = Control Group; I1 = Intervention Group 1; I2 = Intervention Group 2

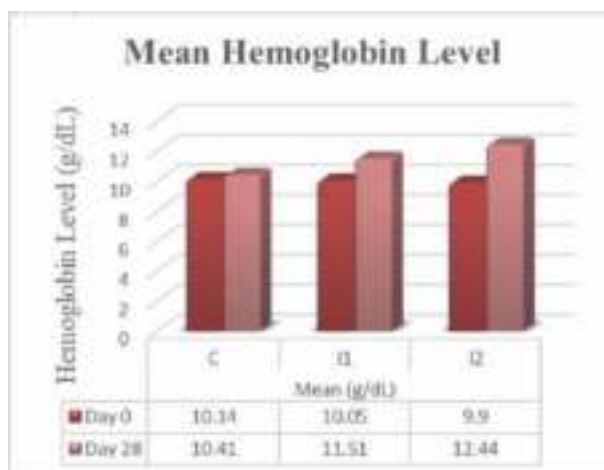


Table 2 and Figure 2 demonstrate that between day 0 and day 28, both intervention group 1 and intervention group 2 experienced a significant increase in hemoglobin levels, while the control group showed an increase in hemoglobin levels, but it is not statistically significant. The most substantial increase in hemoglobin levels is observed in intervention group 2, followed by intervention group 1, and the smallest increase is in the control group. The nonsignificant increase in hemoglobin levels in the control group suggests that providing iron supplementation alone may not lead to a significant improvement in hemoglobin levels in adolescent girls experiencing moderate anemia. This finding is inconsistent with the study by Jalambo *et al.* (2018), which indicated that consuming iron supplements could increase hemoglobin levels in the blood. This is because iron deficiency conditions can stimulate increased iron absorption in anemic subjects. Firstly, the absorbed iron can be utilized to normalize hemoglobin levels, ensuring that the supply of oxygen to tissues/cells is sufficient (Susanti *et al.*, 2016). This means that providing iron supplementation alone may not effectively contribute to tissue/cell oxygenation.

The statistical test results in this study indicate that hemoglobin levels in intervention group 1 and intervention group 2 are significantly higher than those in the control group. This implies that cava smoothies, made from bananas, avocados, honey, and dates, when combined with iron supplementation, can significantly increase hemoglobin levels. As it is known from laboratory test results of its nutritional content, cava smoothie contains iron and vitamin C, among other nutrients. The presence of iron content can increase hemoglobin levels because iron is a component of the formation of hemoglobin (Abbaspour *et al.*, 2014). Additionally, the presence of vitamin C content will also increase hemoglobin levels because vitamin C aids in the absorption of iron from food/drinks, allowing it to be processed into red blood cells once again (Chavan *et al.*, 2021).

The statistical test results also indicate that the increase in hemoglobin levels in intervention group 2 is significantly higher compared to intervention group 1. Furthermore, in group I2, after receiving iron supplementation and cava smoothie, the

mean hemoglobin level shows a normal value of 12.2 ± 0.6 mg/dL, while in group I1, the mean hemoglobin level is still below normal. This means that an increased dosage of cava smoothie can provide a more optimal increase in hemoglobin levels. The results of this study align with research conducted by Tuju *et al.* (2019), which showed that a combination of 200 g of bananas and 60 mg of iron supplementation for 7 days could significantly increase hemoglobin levels compared to iron supplementation alone. Another study conducted by Utami (2020), also indicates that the combination of 100 g of avocado and 60 mg of iron supplementation for 7 days can result in a higher increase in hemoglobin levels compared to iron supplementation alone. Damayanti *et al.* (2021) also researched the combination of 1 tablespoon of honey and 60 mg of iron supplementation for 14 days, resulting in a higher increase in hemoglobin levels than 60 mg of iron supplementation alone. Additionally, the study by Novadela & Imron (2015) showed that the combination of 2 dates and 60 mg of iron supplementation for 21 days could lead to a higher increase in hemoglobin levels compared to 60 mg of iron supplementation alone.

Bananas contain vitamin C, vitamin B1, energy, fat, carbohydrates, calcium, phosphorus, iron, and water (Ruspita *et al.*, 2022). The iron content in bananas can stimulate hemoglobin production in the blood (Adethia & Sukarni, 2022). The vitamin C content in bananas can accelerate the absorption of iron and help in the treatment of anemia patients (Mahardika & Zuraida, 2016). Consuming avocados, which are rich in iron and vitamin C, is very beneficial for the growth of red blood cells in the body and can help prevent and treat anemia, thus addressing complications resulting from anemia (Kiswari, 2018). Honey is a sweet-tasting herbal substance produced by honeybees from the nectar of flowers or liquids derived from plant matter, which is collected, modified, and combined into a positive compound by bees. The mineral magnesium content in honey is similar to the magnesium content in human blood serum. Similarly, the iron content in honey can increase the number of red blood cells in human blood and enhance hemoglobin levels (Panjaitan, 2018). Dates are a high-energy

source of nutrition with an ideal composition. They contain carbohydrates, tryptophan, omega-3, vitamin C, vitamin B6, Ca2, Zn, and Mg, and are rich in fiber. Additionally, they contain potassium, manganese, phosphorus, iron, sulfur, calcium, and magnesium.

The increase in hemoglobin levels occurs after the consumption of dates (As *et al.*, 2021).

Giving Cava Smoothie and iron supplementation increases red blood cell indices (MCV, MCH, MCHC).

Table 3. Mean MCV (Mean Corpuscular Volume) on Day 0 and Day 28, and the Difference in Each Group

Group	Mean ± SD (fL)			Kruskal-Wallis Test
	C	I1	I2	
Day 0	79.05 ± 3.25	78.97 ± 6.47	78.82 ± 1.99	0.127
Day 28	79.40 ± 3.01	79.67 ± 6.50	80.79 ± 1.67	0.010*
▲ (fL)	0.35 ± 0.64 ^a	0.69 ± 2.12 ^b	1.97 ± 1.19 ^c	0.001*
Wilcoxon Test	0.120	0.001*	0.001*	

Note: C = Control Group (given iron supplementation 60 mg/week); I1 = Intervention Group 1 (given iron supplementation 60 mg/week and cava smoothie 100 ml/every 2 days); I2 = Intervention Group 2 (given iron supplementation 60 mg/week and cava smoothie 200 ml/every 2 days); a, b, c = Significantly different in Mann-Whitney Test; * = Significantly different (<0.05).

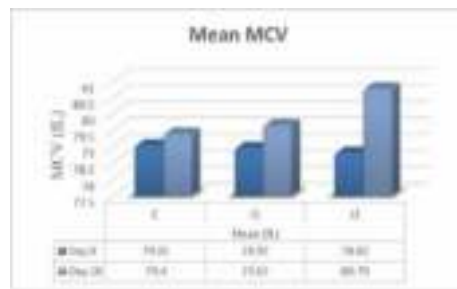


Figure 3. Bar Chart of Mean MCV on Day 0 and Day 28 in Each Group
 Legend: C = Control Group; I1 = Intervention Group 1; I2 = Intervention Group 2.

Table 4. Mean MCH (Mean Corpuscular Hemoglobin) on Day 0 and Day 28 and the Difference in Each Group

Group	Mean ± SD (pg)			Kruskal-Wallis Test
	C	I1	I2	
Day 0	25.13 ± 0.69	25.05 ± 0.68	24.90 ± 0.77	0.616
Day 28	25.47 ± 0.39	25.66 ± 0.58	26.51 ± 0.84	0.001*
▲ (pg)	0.34 ± 0.62 ^a	0.61 ± 0.69 ^b	1.61 ± 0.99 ^c	0.001*
Wilcoxon Test	0.050	0.010*	0.010*	

Note: C = Control group (given iron supplementation 60 mg/week); I1 = Intervention group 1 (given iron supplementation 60 mg/week and cava smoothie 100 ml/every 2 days); I2 = Intervention group 2 (given iron supplementation 60 mg/week and cava smoothie 200 ml/every 2 days). a, b, c = Significantly different in Post Hoc Test, * = Significantly different (<0.05).



Figure 4. Bar Chart of Mean MCH on Day 0 and Day 28 in Each Group
 Legend: C = Control Group; I1 = Intervention Group 1; I2 = Intervention Group 2

Table 5. Mean MCHC (Mean Corpuscular Hemoglobin Concentration) on Day 0 and Day 28 and the Difference in Each Group

Group	Mean \pm SD (g/dL)			Kruskal-Wallis Test
	C	I1	I2	
Day 0	32.13 \pm 0.59	32.00 \pm 0.68	31.88 \pm 0.77	0.562
Day 28	32.40 \pm 0.31	32.64 \pm 0.60	33.48 \pm 0.83	0.001*
▲ (%)	0.27 \pm 0.55 ^a	0.64 \pm 0.66 ^b	1.60 \pm 0.99 ^c	0.001*
Wilcoxon Test	0.090	0.010*	0.010*	

Note: C = Control Group (given iron supplementation 60 mg/week); I1 = Intervention Group 1 (given iron supplementation 60 mg/week and cava smoothie 100 ml/every 2 days); I2 = Intervention Group 2 (given iron supplementation 60 mg/week and cava smoothie 200 ml/every 2 days); a, b, c = Significantly different in Mann-Whitney test; * = Significantly different (<0.05).

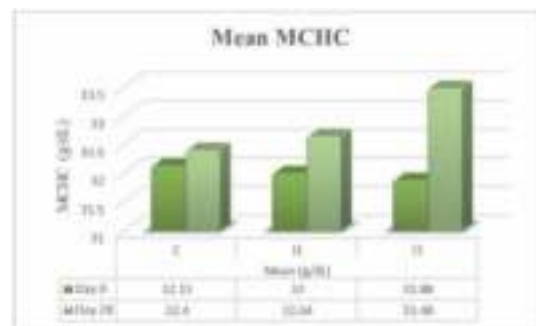


Figure 5. Bar Chart of Mean MCHC on Day 0 and Day 28 in Each Group
Legend: C = Control Group; I1 = Intervention Group 1; I2 = Intervention Group 2.

Tables 3, 4, and 5, as well as Figures 3, 4, and 5, indicate that between day 0 and day 28, both intervention groups 1 and 2 experienced a significant increase in MCV, MCH, and MCHC, while the control group showed an increase that was not statistically significant. The increase in MCV, MCH, and MCHC was most pronounced in intervention group 2, followed by intervention group 1, and was smallest in the control group. The non-significant increase in MCV, MCH, and MCHC in the control group indicates that iron supplementation alone may not significantly improve MCV, MCH, and MCHC in adolescent girls with moderate anemia. This finding is inconsistent with the study conducted by Zhang *et al.* (2020), which found that iron supplementation can significantly increase MCV in the blood. Dissimilar results were also found in a study conducted by Wahyuni (2021), which revealed that consuming iron supplements can significantly increase MCH and MCHC in the blood. The statistical analysis results in this study indicate that MCV, MCH, and MCHC in intervention group 1 and intervention group 2 are significantly higher than in the control group. This means that cava smoothies made from bananas, avocados,

honey, and dates, when combined with iron supplementation, can significantly increase MCV, MCH, and MCHC levels. As known, based on the laboratory analysis of nutritional content, cava smoothie contains, among other things, iron and vitamin C. Iron and vitamin C are best consumed together. Vitamin C assists the body in absorbing non-heme iron by binding to it and helping it flow into the intestines. When vitamin C binds with non-heme iron, it enhances its stability and solubility. This allows the body to more easily absorb iron through the intestinal mucosa (Piskin *et al.*, 2022).

The statistical results also indicate that the increase in MCV, MCH, and MCHC in intervention group 2 is significantly higher compared to intervention group 1. This means that an increase in the dosage of cava smoothie can provide a more optimal effect on MCV, MCH, and MCHC. In this study, cava smoothie was administered over a period of 28 days with a graded dosage, namely 100 ml every 2 days and 200 ml every 2 days. MCV, or Mean Corpuscular Volume, serves to measure the average size of red blood cells, and if MCV is low, it indicates that the red blood cells are very small. MCH and MCV are directly related;

if MCV increases, MCH will also increase, and if MCV is low, MCH will be low as well (Peng *et al.*, 2021). MCH is directly proportional to MCHC; if MCH increases, then MCHC will also increase. The increase in MCHC occurs due to the improving condition of iron-deficiency anemia (Fitriany & Saputri, 2018).

CONCLUSION AND RECOMMENDATIONS

It can be concluded that giving Cava Smoothie and iron supplementation is effective in increasing hemoglobin levels and red blood cell indices (MCV, MCH, and MCHC) in anemic adolescent girls ($p < 0.05$). However, further research is needed to examine parameters for anemia beyond hemoglobin levels and red blood cell indices, such as hepcidin and serum ferritin.

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NUTRACEUTICAL POTENTIAL OF ENCAPSULATED PURPLE OKRA (*Abelmoschus esculentus* L. Moench) EXTRACT

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ABSTRACT

The accumulation of free radical production impacts on the development of degenerative diseases which are the highest cause of morbidity and mortality in the world. Biofortification of purple okra in the form of encapsulated extract has the potential as a nutraceutical through the role of antioxidants. This study aimed to analyze the potential of encapsulated purple okra extract as a nutraceutical by determining physical-chemical characteristics, microbial and heavy metals contamination, antioxidant capacity, total flavonoids, and quercetin. This was a laboratory experimental study on purple okra which was extracted using the maceration method. The results of physical-chemical characteristics showed that purple okra extract has the form of dry powder, greenish-brown color, odor characteristic of okra extract (caramel-like), and sour taste with yield of 4%, pH of 4.8, undetectable solvent residue, water content of 13.5%, total ash content of 10.4%, and disintegration time of 1.25 minutes. The results of microbial contamination showed a total plate count of 3.1×10^2 CFU g^{-1} , yeast and mold count, *E. coli*, *S. aureus* negative CFU g^{-1} , and *Salmonella* spp negative CFU $10g^{-1}$. The results of heavy metal contamination showed that As, Pb, Cd, and Hg were not detected every mg Kg^{-1} . The results of antioxidant capacity, total flavonoids, and suspected quercetin derivatives showed a value of 84.88%, 81.32 mg QE g^{-1} , and 4.91 mg g^{-1} . These bioactive components act as free radical scavengers in helping to prevent chain reactions. Encapsulated purple okra extract has shown its potential as a nutraceutical that helps prevent degenerative diseases.

Keywords: antioxidant, encapsulated, nutraceutical, purple okra extract

INTRODUCTION

The prevalence of degenerative diseases is estimated to continue to increase and become the main cause of death globally (Ramesh & Kosalram, 2023). The accumulation of free radicals has an impact on the development of various degenerative diseases through various mechanisms (Sharifi-Rad et al., 2020). Free radicals form reactive oxygen species (ROS) which have one or more unpaired electrons. When the production of free radicals exceeds the limit of protective capabilities, it will initiate an autocatalytic reaction to induce damage to the main components of cells, namely protein, lipid, and deoxyribonucleic acid (DNA) (Burgos-Morón et al., 2019). This condition is the beginning of oxidative stress due to an imbalance between free radical production and natural radical scavengers (Salsabila et al., 2022).

Exogenous antioxidant sources from food or parts of food are needed when the production of endogenous antioxidants as natural radical

scavengers is unable to compensate for the increase in free radicals (El-Masry & Mahmoud, 2021). The availability of exogenous antioxidants can restrain the use of endogenous antioxidants, thereby synergistically increasing defense against oxidative stress (Moussa et al., 2019). Antioxidants work by giving one electron to oxidant compounds so that their activity can be inhibited (Hurrell & Hsu, 2017). Okra is starting to develop in Asia including Indonesia which has been identified as having antioxidant activity with considerable market potential (Bawa & Badrie, 2016). Biofortification of purple okra (*Abelmoschus esculentus* L. Moench) developed by the Center for Tropical Horticulture Studies, IPB University has bioactive components in the form of flavonoid and quercetin which are superior to green okra. Purple okra is the result of developing superior seeds from okra varieties (Anjani, 2018).

The bioactive components in purple okra can be utilized in the form of encapsulated extract.

The encapsulated form facilitates use, controls the release of its active substances at the right target, and protects against external factors to maintain functional stability during storage (Nining et al., 2017; Suwaris & Saputra, 2020). Purple okra is extracted using a maceration method to extract bioactive components so that it has a higher content than the fresh form of okra (Achmad, 2022). The vacuum pan evaporator technology is used to dry the extract with the consideration that reducing pressure can reduce the boiling point so that the temperature is relatively lower and the time is relatively shorter to maintain the bioactive components (Syakdani et al., 2019).

The potential of encapsulated purple okra extract as a nutraceutical was identified through the role of antioxidants as free radical scavengers in helping to prevent chain reactions thereby preventing further damage to cell components (Yunanto et al., 2009). Research by Elkhalfifa et al. (2021) regarding the potential of okra as a nutraceutical for health applications, showed its benefits as an antioxidant, antidiabetic, antihyperlipidemic, antiproliferative, and anticancer. Nutraceuticals, a combined term between nutrition and pharmaceuticals, are food or parts of food that provide added value to improve and enhance the body's physiological functions (Siddiqui & Moghadasian, 2020). This research aims to analyze the potential of encapsulated purple okra extract as a nutraceutical by determining physical-chemical characteristics, microbial and heavy metal contamination, as well as bioactive components that help prevent and reduce the consequences of degenerative diseases.

METHODS

This research used a laboratory experimental design which began with the production of encapsulated purple okra extract at the Pilot Plant, Department of Food Science and Technology, IPB University. The research continued with the analysis of physical-chemical characteristics, microbial and heavy metal contamination, and bioactive components (antioxidant capacity, total flavonoids, and quercetin) at Saraswanti Indo Genetech Laboratory, Bogor and Analysis of Nutrients and Biochemistry Laboratory,

Department of Community Nutrition, IPB University.

Encapsulated Purple Okra Extract Production

Purple okra is the result of the biofortification of okra varieties located at the Leuwikopo Experimental Garden which was developed by Prof. Dr. Muhamad Syukur, S.P., M.Sc. from the Center for Tropical Horticulture Studies, IPB University. Biofortification is carried out by including nutritional elements to produce okra varieties with superior bioactive components.

The production of encapsulated purple okra extract using a modification of research by Fan et al. (2014) and Achmad (2022) is presented in Figure 1. Fresh purple okra was blanched at 100 °C for 1 minute and mashed for 2 minutes. The extraction process was carried out using the maceration method at room temperature for 3×24 hours. Extraction results were dried using a vacuum pan evaporator at 60 °C for 30 minutes. The dry extract was powdered for 10 seconds and packed into capsules.

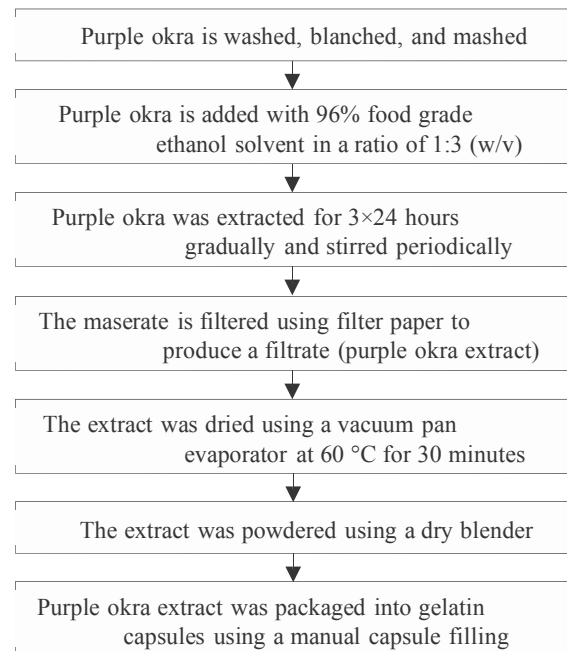


Figure 1. Production Process of Encapsulated Purple Okra Extract

Physical-Chemical Characteristics Analysis

Analysis of the physical-chemical characteristics of purple okra extract in the form

of organoleptic observations was carried out descriptively regarding texture, taste, odor, and color (BPOM, 2019). Yield analysis was carried out by the weighing method using an analytical balance (Depkes, 2000). pH analysis was carried out by the electrometric method using a pH meter (Vernanda et al., 2019).

Solvent residual analysis was carried out by the chromatographic method using gas chromatography according to the United States Pharmacopeia (USP) (2020a). Water content analysis was carried out by gravimetric method using an oven according to SNI 01-2891-1992 5.1 (BSN, 1992a). Total ash content analysis was carried out by the gravimetric method using a furnace according to SNI 01-2891-1992 6.1 (BSN, 1992b). Disintegration time analysis was carried out using a disintegration tester on encapsulated purple okra extract according to the Indonesian Pharmacopeia VI (Kemenkes, 2020).

Microbial Contamination Analysis

Analysis of microbial contamination in the form of total plate count (TPC), yeast mold count (YMC), and the specific microorganism *Escherichia coli* (*E. coli*) was carried out by the microorganism enumeration method using the pour plate technique which was counted using a colony counter according to SNI ISO 4833-1:2015 (BSN, 2015) for TPC, USP (2020b; 2020d) for YMC, and SNI ISO 16649-2:2016 (BSN, 2016) for *E. coli*. *Salmonella spp* analysis was carried out qualitatively using the inoculation method which was confirmed by biochemical test and serological test according to USP (2020b; 2020c). Analysis of *Staphylococcus aureus* (*S. aureus*) was carried out qualitatively using the inoculation method which was confirmed by the staining test and coagulation test according to USP (2020b; 2020c).

Heavy Metal Contamination Analysis

Analysis of heavy metal contamination such as arsenic (As) and lead (Pb) was carried out by the absorbance method using an inductively coupled plasma-mass spectrometer (ICP-MS) according to AOAC 2011.19 (AOAC, 2014), AOAC 2015.01 (AOAC, 2015), and Creed et al. (1994). Analysis of cadmium (Cd) and mercury (Hg) was carried

out by the absorbance method using inductively coupled plasma-optical emission spectrometry (ICP-OES) according to AOAC 2011.14 (AOAC, 2011), AOAC 2013.06 (AOAC, 2013), and Gomez et al. (2007).

Antioxidant Capacity Analysis

Antioxidant capacity analysis was carried out by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method using a microplate reader based on a modification of research by Molyneux (2004) and Khan et al. (2022). A sample of 0.01 g was dissolved with ethanol p.a in a 10 mL volumetric flask. Vitamin C standard of 0.1 g was dissolved with distilled water in a 10 mL volumetric flask. Vitamin C standard solution is diluted at a concentration of 10.000-0.001 ppm, while the sample solution is diluted at a concentration of 1000-31.25 ppm. A total of 150 μ L of each sample and the standard solution was added to 150 μ L of 0.2 mM DPPH solution and then homogenized. The solution was incubated for 30 minutes then the absorbance was measured at a wavelength of 515 nm and the antioxidant capacity was stated in %inhibition.

Total Flavonoids Analysis

Analysis of total flavonoids was carried out by the aluminum chloride (AlCl_3) method using a microplate reader based on a modification of research by Pascal et al. (2018). A sample of 0.01 g was dissolved with ethanol p.a in a 10 mL volumetric flask. Quercetin standard of 0.01 g was dissolved with ethanol p.a in a 10 mL volumetric flask. Quercetin standard solution is diluted at a concentration of 500-15.625 ppm. A total of 500 μ L of each sample and the standard solution was added 30 μ L of 5% NaNO_2 then homogenized and incubated for five minutes. A total of 30 μ L of 10% AlCl_3 solution was added then homogenized and incubated for one minute. A total of 200 μ L of 5% NaOH solution and 240 μ L of distilled water were added and then homogenized. Absorbance was measured at a wavelength of 492 nm. Total flavonoids are calculated through the equation $y = 0,0016x + 0,025$ and stated in mg quercetin equivalent (QE) g^{-1} .

Quercetin Analysis

Quercetin analysis was carried out by a chromatographic method using high-performance liquid chromatography (HPLC) based on a modification of research by Seo et al. (2021). A sample of 0.1 g was dissolved with HPLC-grade methanol in a 10 mL volumetric flask. The sample solution was filtered using a 0.45 μm nylon syringe filter. Quercetin standard of 0.0025 g was dissolved with the same solvent in a 10 mL volumetric flask. A total of 60 μl of sample filtrate was injected into the HPLC system. A low-pressure gradient system with methanol and water as mobile phases was used at ratios of 100:0 (3 minutes), 95:5 (3 minutes), and 90:10 (3 minutes) at a wavelength of 370 nm.

RESULTS AND DISCUSSION

Physical-Chemical Characteristics

The results of the physical-chemical characteristics analysis of encapsulated purple okra extract based on BPOM standard (2019) in encapsulated powder form, Depkes standard (2008), and Depkes standard (2000) in powder extract form are presented in Table 1. Organoleptic observation aims to provide a simple objective introduction by the researchers which is described in the form of dry powder, greenish-brown color, odor characteristic of okra extract (caramel-like), and sour taste. The results of the yield analysis of 4.0% show a different value compared to the standard yield according to Depkes (2000) of $\geq 7.2\%$. The yield provides an estimate of the bioactive components that can be extracted from the extraction process.

The results of the pH analysis show a value of 4.8. This is different from okra flour which is dried using a cabinet dryer which has a pH value of 6.35 (Fauza et al., 2019). The extraction process can lower the pH and lower pH values contain higher antioxidant activity (Rifkowitz & Wardanu, 2016). At low pH, the density of hydrogen increases which suppresses the release of hydrogen to scavenge free radicals (Fathinatullabibah et al., 2014). The residual solvent content after evaporation can be determined through residual solvent analysis to ensure the safety of the extract for consumption. The results show that solvent

Table 1. Physical-Chemical Characteristics of Encapsulated Purple Okra Extract

Parameter	Unit	Purple Okra Extract	Standard
Organoleptic			
Texture		dry powder	-
Color		greenish-brown	-
Odor		okra extract	-
Taste		sour	-
Yield	%	4.0	$\geq 7.2^1$
pH		4.8	-
Solvent residue	ppm	not detected	Max. 10.000 ²
Water content	%	13.5	$\leq 10^2$
Total ash content	%	10.4	$\leq 16.6^3$
Disintegration time	minute	1.25	$\leq 30^2$

Source: ¹Depkes (2000). ²BPOM (2019). ³Depkes (2008).

residue is not detected according to BPOM (2019). The vacuum pan evaporator technology is used to dry the extract which can evaporate the solvent at low pressure so that the temperature is relatively lower and the time is relatively shorter to maintain the bioactive components (Syakdani et al., 2019).

Furthermore, the results show a water content of 13.5% which is different from the standard water content according to BPOM (2019) of $\leq 10\%$. The presence of water content because the purple okra extract is hygroscopic which is able to absorb air humidity or water at normal temperature and normal pressure. Total ash content analysis provides an overview of the inorganic or mineral compounds remaining after the ashing process (Hidayati et al., 2018). The results show a total ash content of 10.4% which is different from the standard total ash content according to BPOM (2019) of $\leq 16.6\%$. The lower ash content indicates an optimal extraction process due to the low mineral residue (Khirzin et al., 2019). This is related to the use of solvents that attract more organic compounds than inorganic compounds.

The encapsulated form has an effect if it is first broken down into smaller particles so that it can be absorbed into the digestive tract. Encapsulated purple okra extract requires a disintegration time of 1 minute 15 seconds which shows a value in accordance with BPOM (2019), namely ≤ 30 minutes. The harder the capsule material, the

smaller the porosity, so it becomes more difficult to penetrate and absorb water into the capsule, which affects disintegration time (Sugiyanto et al., 2017).

Microbial and Heavy Metal Contamination

The results of microbial and heavy metal contamination analysis of encapsulated purple okra extract based on BPOM (2019) standards in encapsulated powder form are presented in Table 2. Microbial contamination analysis can provide assurance that microbial contamination does not exceed the specified limits to ensure the safety of the extract for consumption. The presence of microbes can affect the stability during storage and the safety of the extract. The result of TPC analysis shows a value of 3.1×10^2 colony-forming units (CFU) g^{-1} according to BPOM (2019) of $\leq 2 \times 10^4$ CFU g^{-1} . In addition, YMC analysis as well as specific microorganisms such as *E. coli*, *Salmonella spp.*, and *S. aureus* showed negative results. These results are in accordance with BPOM (2019), namely $\leq 2 \times 10^2$ CFU g^{-1} for YMC, negative g^{-1} for *E. coli* and *S. aureus*, and negative $10g^{-1}$ for *Salmonella spp.* Research on the potential of okra as a nutraceutical shows its benefit as an antimicrobial that can inhibit bacterial growth (Syukri et al., 2020; Elkhalfa et al., 2021).

Table 2. Microbial and Heavy Metal Contamination of Encapsulated Purple Okra Extract

Parameter	Unit	Purple Okra Extract	Standard ¹
Microbialcontamination			
TPC	CFU g^{-1}	3.1×10^2	$\leq 2 \times 10^4$
YMC <i>E. coli</i>	CFU g^{-1}	negative	$\leq 2 \times 10^2$
<i>Salmonella spp.</i>	CFU g^{-1}	negative	negative
<i>S. aureus</i>	CFU $10g^{-1}$	negative	negative
	CFU g^{-1}	negative	negative
Heavy metal contamination			
Arsenic	mg Kg^{-1}	not detected	≤ 5
Lead	mg Kg^{-1}	not detected	10
Cadmium	mg Kg^{-1}	not detected	≤ 0.3
Mercury	mg Kg^{-1}	notdetected	≤ 0.5

Source: ¹BPOM (2019)

Heavy metal contamination analysis can provide assurance that heavy metal contamination does not exceed the specified limits that cause toxicity (Depkes 2000). The results show that heavy metal contamination such as As, Pb, Cd, and Hg are not detected according to BPOM (2019) in units of mg Kg^{-1} , namely ≤ 5 for As, 10 for Pb, ≤ 0.3 for Cd, and ≤ 0.5 for Hg. Heavy metal contamination can occur during the production of purple okra extract. In addition, industrial waste or agricultural activities such as the geological condition of the land where cultivation is carried out and the fertilizer used also affect contamination (Wijianto et al., 2022). Heavy metal contamination provides negative effects which are mostly mediated through increased production of excess ROS causing oxidative damage to various body's physiological systems (Awoke et al., 2020).

Antioxidant Capacity

The ability of antioxidant compounds in okra extract to inhibit free radicals is known as antioxidant capacity (%inhibition). The results of antioxidant capacity analysis showed that the %inhibition of purple okra extract was 84.88% (Table 3). The results of this study are different from the results of research by Zainuddin et al. (2022) on the ethanol extract of green okra which has an inhibition of 81.9%. The difference occurs because purple okra is the result of biofortification which has superior bioactive components compared to green okra (Anjani et al., 2018). Furthermore, the difference can also occur because the previous study used dry okra which was extracted and concentrated using a rotary evaporator at <50 °C to obtain a thick extract. In contrast to this study, which used fresh okra before the extraction process and vacuum pan evaporator technology to dry the extract. This technology works at a pressure of 65 cmHg so that the boiling point of the solvent can be lowered and the time required is relatively shorter (Syakdani et al., 2019). Heat treatment and light exposure for a certain time affect antioxidant compound that has thermolabile properties by triggering pre-oxidation (Rifkowaty & Wardanu, 2016).

Table 3. Antioxidant Capacity, Total Flavonoids, and Quercetin of Encapsulated Purple Okra Extract

Parameter	Unit	Purple Okra Extract
Antioxidant capacity	%	84.88
Total flavonoids	mg QE g ⁻¹	81.32
Quercetin	mg g ⁻¹	4.91

This is different from research by Anjani (2018) on green okra and purple okra in extract form which has an inhibition of 19.28% and 23.34%. Apart from being caused by differences in varieties and forms of dried okra before extraction, the difference in inhibition was also caused by the solvents used. In the previous study, okra was extracted using methanol, while in this study okra was extracted using ethanol. Research shows that the use of ethanol as a solvent shows higher amounts of bioactive components (Padmawati et al., 2020). Antioxidant compounds such as phenol are polar so using ethanol as a solvent is appropriate. Another study showed a different inhibition of 76.28% in okra fruit extract compared to okra leaf extract of 62.12% (Faisal & Handayani, 2019). This is because the fruit contains seeds and mucilages which are known to contain more antioxidant compounds, especially quercetin derivatives in the form of isoquercitrin (Chaemsawang et al., 2019).

Encapsulated purple okra extract with antioxidant content has the potential as a nutraceutical that can increase the capacity to withstand oxidative stress. Antioxidants can prevent, inhibit, eliminate, or repair oxidative damage to target molecules that occur as a result of chemical reactions involving free radicals (Banjarnahor & Artanti, 2014). Several mechanisms of antioxidant action include inhibition of enzymes involving the formation of ROS, termination of radical chain reactions, stabilization of initiator radicals, and enhancement of endogenous antioxidants (Sachdeva et al., 2014).

In addition, several studies show the health benefits of bioactive components of purple okra, namely increasing antioxidant status, improving oxidative stress conditions, hypoglycemia effects

(Anjani et al., 2018; Nabila et al., 2018), improving lipid profiles (Nabila et al., 2018), improving kidney functions (Wahyuningsih et al., 2021a), improving liver functions (Wahyuningsih et al., 2021b), anticancer effects (Achmad, 2022), and reducing inflammation (Pramudya et al., 2022).

Total Flavonoids

The bioactive component of flavonoids in purple okra extract acts as an antioxidant to repair damage caused by radical compounds (Nabila et al., 2018). The results of the total flavonoids analysis showed a value of 81.32 mg QE g⁻¹ (Table 3). In contrast to research on green okra extract by Chandra et al. (2022) with total flavonoids of 3.19 mg QE g⁻¹. Apart from being caused by differences in okra varieties, the previous study required maceration for 5 days, while this study required 24 hours only. Maceration time of 24 hours has higher bioactive components compared to 12, 36, 48, 60, and 72 hours (Widodo et al., 2021). The longer the maceration time tends to reduce the total flavonoids due to oxidation which damages the flavonoid compounds after the optimum time has passed. The total flavonoids were also different compared to the research by Syam et al. (2020) in extracts from green okra and red okra of 2.57 mg QE g⁻¹ and 2.84 mg QE g⁻¹. Apart from being caused by differences in okra varieties, previous study used a different solvent concentration, namely 70%, while this research used 96%. Solvent concentration can result in changes in solvent polarity, thereby affecting the solubility of bioactive compounds (Suhendra et al., 2019).

Encapsulated purple okra extract has the potential as a nutraceutical with flavonoid content which plays a role not only in improving health status but helps prevent and reduce the consequences of degenerative diseases (Balentine et al., 2015). Flavonoid prevent damage by activating the main antioxidant defense enzymes, activating metal chelators, and inhibiting the activity of enzymes that produce free radicals (Banjarnahor & Artanti, 2014; Panche et al., 2016). According to Procházková et al. (2011), flavonoids easily donate hydrogen atoms to radical compounds resulting in the reduction of highly oxidized radicals. Flavonoid phenoxyl radical can change into aroxyl radical which is capable

of carrying out secondary radical scavenging activities by transferring spare electrons and obtaining a stable structure.

Quercetin

Quercetin is one of the main flavonols from the class of flavonoids which has a hydroxyl group on the benzo-dihydropyran ring which makes quercetin have strong antioxidant activity (Yang et al., 2020). Quercetin can express a higher antioxidant capacity than other flavonoid derivatives (Banjarnahor & Artanti, 2014). The results of the analysis of suspected quercetin derivatives in the encapsulated purple okra extract showed a value of 4.91 mg g⁻¹ (Table 3). The results of the chromatogram using HPLC are shown in Figure 2. The retention time that appeared with the largest area on standard quercetin was 1.213 minutes, while for purple okra extract was 1.111 minutes. Spike results using a quercetin standard on purple okra extract (1:4) showed a new peak that was visible at the retention time of the quercetin standard. Based on the results of identifying quercetin derivatives, it can be assumed that the compound seen in purple okra extract is a quercetin derivative, namely quercetin-3'-O-sulphate (Yang et al., 2018) or quercetin rhamnoside-(feruloyl-hexoside) (Acquavia et al., 2021) with a retention time adjacent to the retention time of quercetin.

The presence of quercetin in okra is qualitatively known but has not been analyzed quantitatively. Green okra is known to contain quercetin of 0.018 mg g⁻¹ (Utami, 2018). The quercetin content was different compared to the green okra extract form in the same study, which was 2.47 mg g⁻¹. The extract form can attract bioactive components so that it has a higher quercetin content. When compared to green okra, purple okra has a different quercetin content of 0.039 mg g⁻¹ in fresh form (Utami, 2018) and 0.45 mg g⁻¹ in extract form (Anjani, 2018). Biofortification of purple okra produces varieties with superior bioactive components (Anjani, 2018).

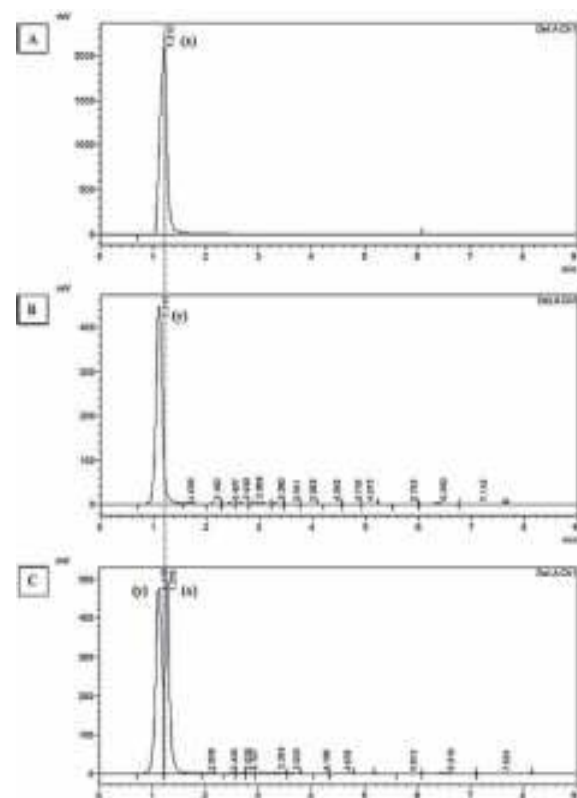


Figure 2. Quercetin Analysis Chromatogram. (A) Quercetin Standard, (B) Purple Okra Extract, (C) Spike of Quercetin Standard and Purple Okra Extract, (x) Quercetin Standard Peak, (y) Suspected Quercetin Derivative Peak

Encapsulated purple okra extract has potential as a nutraceutical with quercetin content which can improve oxidative stress conditions. According to Xu et al. (2019) and Yang et al. (2020), quercetin has effects on signal transduction pathways such as activating, inhibiting, increasing, and downregulating conditions related to oxidative stress. The antioxidant activity of quercetin has been identified primarily through its effect on regulating glutathione peroxidase levels and increasing the expression levels of endogenous antioxidant enzymes. Quercetin also works with its ability to scavenge free radicals including superoxide anions, hydroxyl radicals, and peroxy radicals as well as better inhibition of ROS formation compared to other classes of flavonoids (Demirci, 2017; Elkhailifa et al., 2021).

CONCLUSION

Encapsulated purple okra extract shows its potential as a nutraceutical which has a dry powder form, greenish-brown color, odor characteristic of okra extract (caramel-like), and sour taste with yield of 4%, pH of 4.8, undetectable solvent residue, water content of 13.5%, total ash content of 10.4%, and disintegration time of 1.25 minutes. Microbial contamination showed TPC of 3.1×10^2 CFU g⁻¹, YMC, *E. coli*, and *S. aureus* negative CFU g⁻¹, and *Salmonella spp* negative CFU 10g⁻¹. Heavy metal contamination showed As, Pb, Cd, and Hg contamination not detected per mg Kg⁻¹. Bioactive components such as antioxidant capacity, total flavonoids, and suspected quercetin derivatives showed values of 84.88%, 81.32 mg QE g⁻¹, and 4.91 mg g⁻¹. These bioactive components act as free radical scavengers in helping prevent and reduce the consequences of degenerative diseases. Further research can prove in vivo the potential of encapsulated purple okra extract as a nutraceutical.

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PHYSICOCHEMICAL CHARACTERISTICS, ANTIOXIDANT ACTIVITY AND SENSORY OF COOKIES BASED ON MOCAF, PURPLE YAM, AND CINNAMON FLOUR

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ABSTRACT

In general, cookies are made by using wheat flour with high gluten content, consequently it may cause negative impact on health. So far, cookies also have not the characteristics as functional food that is beneficial for health. Based on these issues, wheat flour is needed to substitute with alternative materials in order to reduce the gluten and increase the functional characteristic of cookies. Mocaf, purple yam flour and cinnamon powder can be used to provide cookies with low gluten and have the characteristic of functional food. The aims of this research are to characterize the physicochemical and organoleptic properties of cookies based Mocaf flour, purple yam flour and cinnamon powder. This work was used he completely randomized design (CRD) with the formulations applied F₀(100:0:0), F₁(75:24.5:0.5), F₂(75:24:1), F₃(75:23.5:1.5), F₄(50:49.5:0.5), F₅(50:49:1), F₆(50:48.5:1.5), F₇(25:74.5:0.5), F₈(25:74:1), and F₉(25:73.5:1.5) with 3 replications. Analysis of physicochemical properties of cookies consists of moisture, fat, total protein, ash, carbohydrates contents, texture, and antioxidant activity. The organoleptic properties were tested by 30 semi-trained panelists. The collected data were analyzed by one way ANOVA at significance level of 5% and followed by Duncan's test. These results show the contents of fat, protein, ash, carbohydrate, water, antioxidant activities and hardness were obtained in the range of 28.1-29.4%, 3.3-3.6%, 1.5-2.2%, 59.8-62.1%, 44.5-88.8 ppm and 13.8 - 38.3 N, respectively. Based on the organoleptic tests, F₂ cookies was most preference than other formulations. The cookies produced believes have low gluten and food functional properties

Keywords: cinnamon, cookies, mocaf, purple yam flour

INTRODUCTION

Public health is the most important factor in supporting the progress of a nation. Good public health conditions play an important role in increasing productivity, education, development, social stability, and security as well as the development of human resources. In Indonesia, the management of health problems is regulated in the Regulation of the Minister of Health of the Republic of Indonesia Number 21 of 2020 concerning health management strategy plans (Putranto, 2020). Health management is comprehensive and reaches all levels of society so that health conditions are more under control.

Currently, several health issues such as celiac and degenerative diseases. Celiac disease is an autoimmune process in the body that is triggered by the habit of consuming foods high in gluten. The body gives an autoimmune reaction to

gluten so that it interferes with the intestines and absorption of nutrients, causes gastrointestinal symptoms, and others. If this disease is not treated properly it can result in complications of various other types of diseases (Oktadiana, Abdullah, & Renaldi, 2017). Meanwhile, degenerative diseases such as heart disease, diabetes mellitus are also very important to overcome.

A good method for overcoming and preventing these diseases is to adopt a healthy diet and get used to consuming functional foods. Functional food is food that has basic nutrition and has a positive effect on health. The ingredients used in the manufacture of functional food must contain basic nutrients and bioactive components that are beneficial to health. Functional food can be prepared using a variety of basic ingredients such as vegetables, fruits, grains, tubers, herbs, and others (Suter, 2013).

One of the food products that can be innovated into functional food products is cookies. Cookies are crispy dry bread with a sweet taste and are usually made from main ingredients such as wheat flour or wheat flour (Herawati, Suhartatik, & Widanti, 2018). Currently, cookies are growing and are available in various variants such as chocolate, peanut, coconut, vanilla, red velvet, blueberry, and others. However, the main ingredients used for making cookies are the same, namely wheat flour or wheat. As it is known that wheat flour (especially high in gluten) is not good for health. Gluten is a protein component composed of gliadin (20-25%) and glutenin (35-40%) (Fitasari, 2009) which are mostly found in cereal foods such as wheat flour (F Kusnandar, Harya, & Agus, 2022)

Apart from the negative impact on health, another disadvantage of high-gluten wheat flour is that it produces dough for cookies that is tough and tough (Masrikhiyah, 2021). Also, the resulting cookie products are not suitable for consumption by people with celiac disease. Patients with celiac disease such as anemia, osteoporosis, dermatitis herpetiformis, neurological symptoms, and diabetes mellitus (Gujral, Freeman, & Thomson, 2012) will detect gluten as a dangerous component, due to changes in the small intestine that result in impaired absorption of nutrients into the body (Permatasari, Ina, & Yusa, 2018). Therefore, people with celiac disease are advised to reduce the consumption of foods that contain gluten (Gujral et al., 2012).

The concept of gluten-free cookies with the theme of functional food as a new food product innovation is very interesting to develop. Indeed, the nature of functional food is not solely determined by high, low, or no gluten content, however, foods that are high in gluten content generally tend to cause adverse effects on health, so they are not suitable to be called functional foods. The concept of gluten-free cookies can be realized by substituting wheat flour with alternative flour (which does not contain gluten) such as modified cassava flour (Mocaf) (Tanjung & Kusnadi, 2015). In addition, the concept of cookies as functional food can also be realized through the use of raw materials that contain other compounds that can provide benefits to the body, such as antioxidant compounds.

It is well known that apart from being high in gluten, cookies usually do not contain (low) antioxidants. Therefore, innovation is needed to overcome this problem. Foodstuffs that can be used as sources of antioxidants include purple yam and cinnamon because these two types of ingredients are rich in antioxidants. According to research results Prasetyo and Winardi (2020) that the antioxidant activity (based on radical scavenging activity, RSA) in fresh purple yam was around 62.14%, and purple yam flour was around 20.19%. Whereas in cinnamon bark the antioxidant activity (based on the inhibition concentration, IC_{50}) is around 1.94 ppm (Antasionasti & Jayanto, 2021).

Gluten-free and high-antioxidant cookies have several advantages, including being suitable for consumption by people with celiac disease and being beneficial for the body as an antidote to free radicals. Many studies have been reported on the use of Mocaf flour, purple yam flour, and cinnamon powder for making cookies, such as research on making cookies. For example, (Herawati et al., 2018; Rasyid, Maryati, Triandita, Yuliani, & Angraeni, 2020) were used Mocaf flour, Fitriani, Yurnalis, and Hermalena (2019) used purple yam flour and white yam(40:60) and Fairus, Hamidah, and Setyaningrum (2021) were used Mocaf flour, purple yam and peanuts (20:35:45). The physicochemical characteristics of the cookies produced such as moisture, fat, protein, ash, and carbohydrate contents were obtained in the range of 1.1 - 3.1%, 12.7 - 25.2%, 2.9 - 12.6%, 0.9 - 1.1%, and 29.6%, respectively. Meanwhile, the results of research on antioxidant activity in cookies made from purple yam flour and peanuts are reported by Martins, Susilowati, and Jinarti (2014) where the percentage of radical scavenging activity (%RSA) is around 39.7%.

The use of local food commodities can reduce the need for wheat flour (Tamaroh & Sudrajat, 2021). Many local food commodities have not been used optimally, such as cassava (*Manihot esculenta*) and purple yam (*Ipomoea batatas*). Cassava can be processed through fermentation to produce gluten-free flour known as Mocaf. Meanwhile, purple yam (*Ipomoea batatas L.*) can be processed into antioxidant-rich flour (Nabilah, 2019). The use of cinnamon (*Cinnamomum*

burmannii) as an additional ingredient in making cookies is also an interesting innovation because cinnamon is rich in antioxidant compounds such as eugenol, safrole, cinnamaldehyde, tannins (Hariana, 2007), and polyphenol (Priani, Darusman, & Humanisya, 2014).

Parameters of cookie quality involving physicochemical and sensory properties are very important to evaluate before the cookies are commercialized. Quality cookies are cookies that meet physicochemical and sensory quality criteria. In general, the physicochemical properties that are often evaluated include moisture content, protein, fat, ash, carbohydrates and texture. Based on SNI-2973-2018 that the maximum water content is 5%, the minimum protein is 9%, the minimum fat is 9.5%, the maximum ash is 1.5% and carbohydrates is maximum 70% (BSN, 2018). In addition, according to Rahardjo, Nugroho, and Saibele (2021) the sensory also determines the quality of cookies. Sensory parameters evaluated include aroma, taste, color, and texture.

Based on the illustration above, the scenarios are needed to produce gluten-free cookie products that have functional food characteristics. This research tries to the use of Mocaf and purple yam flour as basic ingredients for making cookies, and cinnamon powder as an additional ingredient. The purpose of this study is to determine the physicochemical characteristics, antioxidant activity, and sensory activity of cookies made from Mocaf flour and purple yam flour with added cinnamon powder. In addition, this study also aims to provide information related to the formulation of good cookies based on the nutritional, antioxidant, and sensory aspects. Several physicochemical properties of cookies were analyzed such as moisture content, fat, total protein, ash, carbohydrates, hardness, and antioxidant activity. Meanwhile, the sensory properties of the cookies that were evaluated included color, taste, aroma, texture, and overall preferences.

MATERIALS AND METHODS

Materials and Instruments

The materials were cassava, purple yam and cinnamon. Other materials needed were margarine, sugar, eggs, skimmed milk, and baking powder.

All materials were purchased from the Yogyakarta Traditional Market. The instruments used were a sieve, blender, rolling pin, Soxhlet, UV-Vis Spectrophotometer (Thermoscientific), Oven (Memmert), and UTM (Zwick/z0.5).

Mocaf flour Preparation

The preparation of Mocaf flour was performed by using a method as described by Yani and Akbar (2018). A total of 1 kg of cassava peeled was washed with water and sliced using grated chips. After that, the cassava slices were soaked in water, added a total of 5 g of yeast (*Saccharomyces cerevisiae*), and left for 12 hours. Then, the cassava slices were drained and dried in an oven at 60°C for 24 hours. Dried cassava slices were fined by using a blender for 15 minutes and then sieved through an 80 mesh. The Mocaf flour product was shown in **Figure 1**.



Figure 1. Mocaf flour.

Purple Yam Flour Preparation

The preparation of purple yam flour was performed using a method as described by Anggarawati, Ekawati, and Wiadnyani (2019). A total of 1 kg of sorted purple yam was peeled, washed with clean water, sliced with grated chips, placed on a baking sheet, and dried in an oven at 60°C for 4 hours. Then, the dried slices were fined with a blender and sieved through a 60 mesh. The product of purple yam flour was shown in **Figure 2**.



Figure 2. Purple yam flour

Cinnamon Powder Preparation

The preparation of cinnamon powder was performed as described by Shahid et al. (2018). A total of 75 g of cinnamon bark was washed with clean water, cut into small pieces, and dried in an oven at 60°C for 6 hours. After that, the dried cinnamon pieces were mashed using a blender and sieved through a 60 mesh. The product of cinnamon powder was shown in **Figure 3**.



Figure 3. Cinnamon powder

Experimental Design

This work was designed by a completely randomized design (CRD) with a comparison composition of Mocaf flour (M), purple yam (PY), and cinnamon powder (C). In detail, **Table 1** was presented the research experimental design in this work.

Table 1. Experimental design in this work

Formulation (%)			Symbols	Repetitions		
M	PY	C		1	2	3
100	0	0	F ₀	F ₀₁	F ₀₂	F ₀₃
75	24,5	0,5	F ₁	F ₁₁	F ₁₂	F ₁₃
75	24	1	F ₂	F ₂₁	F ₂₂	F ₂₃
75	23,5	1,5	F ₃	F ₃₁	F ₃₂	F ₃₃
50	49,5	0,5	F ₄	F ₄₁	F ₄₂	F ₄₃
50	49	1	F ₅	F ₅₁	F ₅₂	F ₅₃
50	48,5	1,5	F ₆	F ₆₁	F ₆₂	F ₆₃
25	74,5	0,5	F ₇	F ₇₁	F ₇₂	F ₇₃
25	74	1	F ₈	F ₈₁	F ₈₂	F ₈₃
25	73,5	1,5	F ₉	F ₉₁	F ₉₂	F ₉₃

Cookies Preparation

The composition of the ingredients for making cookies were presented in **Table 2**. Margarine, fine sugar, and egg yolks were mixed by using a mixer for 3 minutes. Then, the mixtures were homogenized by using a mixer for 2 minutes. After that, the mixtures were added with the baking powder, skim milk, Mocaf flour, purple, and cinnamon powder. Again, the mixture was then homogenized with a mixer for 5 minutes. The dough was printed on the brass and baked in the oven at 180°C for 13 minutes (Waisnawi, Yusasrini, & Ina, 2019).

Table 2. Cookies ingredient

Ingredients (g)	Formulations									
	F ₀	F ₁	F ₂	F ₃	F ₄	F ₅	F ₆	F ₇	F ₈	F ₉
Margarine	85	85	85	85	85	85	85	85	85	85
Fine Sugar Egg yolk Baking powder	60	60	60	60	60	60	60	60	60	60
Skimmed milk	10	10	10	10	10	10	10	10	10	10
Mocaf flour	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5
Purple yam flour	15	15	15	15	15	15	15	15	15	15
Cinnamon powder	100	75	75	75	50	50	50	25	25	25
	0	24,5	24	23,5	49,5	49	48,5	74,5	74	73,5
	0	0,5	1	1,5	0,5	1	1,5	0,5	1	1,5

Hardness Test

The texture was tested based on the level of hardness. The hardness level was tested by using the Universal Testing Machine (UTM, Zwick/z0.5). The levels of hardness were measured based on the amount of force needed to break or penetrate the cookies (Bourne, 2002).

Water content measurement

The water content of cookies was determined by using the AOAC (2005) method. The crucible was primarily dried in the oven at 105°C for 1 hour. The cup was then cooled in a desiccator for 15 minutes and then weighed until the weight was constant. A total of 2 g of sample was placed in a cup and dried in the oven at 105°C for 24

hours. After that, the samples were cooled in a desiccator for 15 minutes and then weighed. The water content was determined using the following Equation 1;

$$C_W = \frac{W_1 - W_2}{W_1} \times 100 \text{ g}$$

Where;

C_W = water content (%); W_1 = weight of the cup and sample before dried (g); W_2 = weight of the cup and sample after dried (g)

Fat content measurement

Fat content was determined by using the Soxhlet method as described in the AOAC (2005). The distillation flask (filled with boiling stones) was dried in the oven at 105°C, cooled, and weighed. Then, the distillation flask was filled with 10 mL of petroleum benzene solvent. At the same time, the cookie samples were crushed, weighed, put in an extraction sleeve, and covered with cotton. Then, the Soxhlet apparatus was installed and the casing containing the sample was placed in the sample holder. The extraction process was carried out at 60-70°C for 6 hours. After the extraction process was completed, the solvent was removed using a rotary evaporator. Lastly, fat content was determined using the following Equation 2;

$$C_F = \frac{W_d - W_o}{W_s} \times 100 \text{ g}$$

Where;

C_F = fat content (%); W_d = weight of flasks and boiling stone (g); W_o = weight of fat, flask, and boiling stone (g); W_s = weight of the sample (g)

Measurement of Total Protein Content

Total protein content was determined using the Kjeldahl method as described in the AOAC (2005). Three stages were carried out including the stages of destruction, distillation, and titration. A total of 0.25 g of the cookie sample was crushed, and put into a 100 mL Kjeldahl flask, added 0.7 g of catalyst N (K_2SO_4 : $CuSO_4$, 2:3) and 4 mL of concentrated H_2SO_4 (98%). The sample destruction process was carried out by heating at 410°C for 1 hour in a fume hood until the color of the solution

was changed to clear green. Then, the solution was cooled, added 50 distilled water and 20 mL of 40% NaOH.

The distillation process was carried out at 100°C. The distillate was collected in an Erlenmeyer containing 10 mL of boric acid solution (H_3BO_3 , 2%) and 3 drops of (bromocresol green methyl red, BCG-MR) indicator. Once the color of the distillate was changed from red to blue and the volume reached 40 ml, the distillation process was stopped. Then, the distillate was titrated with 0.01 N HCl until the pink color appeared. Also, the titration process on blank was performed. Record the volume of titrant used to titrate the sample and the blank. Protein levels were determined using the following Equation 3;

$$C_P = \frac{V_2 - V_1 \times F_k}{V_2} \times N \times F_p \times 100 \text{ g}$$

Where;

C_P = protein content (%); V_1 = volume of titrant for blank (mL); V_2 = volume of titrant for sample (mL); N = normality of HCl (0.01 N); F_p = dilution factor, F_k = conversion factor (6,25)

Ash Content Measurement

The ash content was determined by using the procedure of AOAC (2005). The crucible was dried in the oven at 105°C for 1 hour., cooled in a desiccator for 15 minutes, and then weighed until the weight was constant. A total of 2 g of sample was put into a crucible and the burning process was carried out in a furnace at 600°C for 3 hours. Then, the burning process was stopped, and allowed to cool down to 120°C. The crucible was removed from the furnace and cooled in a desiccator for 15 minutes. Finally, the crucible and ashes were weighed. The ash content was calculated using the following Equation 4;

$$C_{Ash} = \frac{W_2 - W_1}{W_2} \times 100 \text{ g}$$

Where;

C_{Ash} = ash content (%); W_1 = weight of crucible and sample (g); W_2 = weight of crucible and ash (g).

Carbohydrate Content Calculation

Analysis of carbohydrate content can be determined based on the difference of 100% of total content minus the water, fat, protein, and ash contents. Equation 5 can be used to calculate carbohydrate content.

$$C_C = 100\% - (W + F + P + Ash)\% \dots (5)$$

Where;

C_C = carbohydrate content (%); W = water content (%); F = fat content (%); P = protein content (%); Ash = ash content (%)

Antioxidant Activity (IC₅₀) Analysis

The procedure for analyzing the antioxidant activity was carried out by using the method as described by Indriyani, Nurhidajah, and Suyanto (2013). The solution of 0.2 M DPPH was prepared by dissolving 0.8 g of DPPH powder (BM 394.32 g/mol) in 10 mL of methanol. Then, a total of 0.1 ml of the 0.2 M DPPH solution was taken and put in a 100 ml volumetric flask, then diluted with methanol to obtain a 0.2 mM DPPH. After that, a total 1 ml of 0.2 mM DPPH solution was taken, put in a test tube, and add 4 ml of methanol, homogenized using a vortex and incubated for 30 minutes. Finally, determine the absorbance of the solution (blank) using a UV-Vis spectrophotometer (Thermo Scientific) at a wavelength of 517 nm.

The sample preparation was carried out by dissolving 10 mg of cookies in 10 mL of methanol in a test tube. Then, the sample solution was prepared in the series concentration of 100 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm, respectively. A total of 1 mL of each sample solution was taken, add 1 mL of 0.2 mM DPPH solution, placed in different test tubes, and diluted with methanol up to 5 mL of total volume. Then, the mixture was homogenized using a vortex and incubated for 30 minutes. Lastly, sample absorption was measured using a UV-Vis spectrophotometer (Thermo Scientific) at a wavelength of 517 nm. The percentage of inhibition was calculated by using Equation 6.

$$\text{Inhibition (\%)} = \frac{A_b - A_s}{A_b} \times 100\% \dots (6)$$

Where;

A_b = absorption of the blank; A_s = absorption of the sample

Antioxidant activity (IC₅₀) was determined by using the calibration curve from the inhibition percentage. The percentage of inhibition was plotted as the y-axis and the \ln of the concentration was plotted as the x-axis so that Equation 6 was obtained. Then, the IC₅₀ was determined using Equation 7.

$$y = ax + b \dots (6)$$

$$50 = ax + b$$

$$x = \frac{50 - b}{a}$$

$$IC_{50} = \text{anti} \ln x \dots (7)$$

Where;

a = slope; b = intercept; x = concentration of antioxidant (ppm)

Sensory Test

The sensory properties of cookies such as color, taste, aroma, texture, and overall level of preference were evaluated by involving 30 untrained panelists. The number of untrained panelists can be selected around 25 - 50 people to get good sensory test results (Meilgaard, Gail Vance Civile, & Carr, 2007). The panelists in this study were selected from healthy students consisting of 15 boys and 15 girls with an average age of 20-21 years. Scoring based on preference levels were 1 (dislike very much), 2 (dislike), 3 (neutral), 4 (like), and 5 (very like).

Data Analysis

The data obtained were analyzed by using SPSS software version 25.0. with a one-way ANOVA test at a significance level of 5% ($\alpha = 0.05$). If there was a significant difference between the treatments, then a further test was carried out using Duncan's test.

RESULTS AND DISCUSSION

Cookies and Their Physicochemical Properties

Overall, the physicochemical characteristics of cookies based on Mocaf flour, purple yam flour, and cinnamon were shown in **Table 3**. As a comparison, some relevant research literature was also presented.

The types of cookie products produced were shown in **Figure 4**. Visually, the cookies showed that the color of the cookies gets darker as

the purple yam flour composition increases. This can be caused by the purple pigment of the purple sweet potato.

Table 3. Physicochemical properties of cookies

Sample	Water content (%)	Fat content (%)	Total Protein (%)	Ash content Abu (%)	Carbohydrate content (%)	Hardness (N)
F0	4,8 ± 0,1 ^a	28,5 ± 0,2 ^{ab}	3,4 ± 0,1 ^b	1,5 ± 0,2 ^a	61,8 ± 0,2 ^{de}	35,3 ± 3,5 ^{de}
F1	4,9 ± 0,1 ^{ab}	28,2 ± 0,2 ^a	3,3 ± 0,0 ^a	1,6 ± 0,1 ^a	62,1 ± 0,1 ^e	21,6 ± 4,2 ^{bc}
F2	4,9 ± 0,1 ^{ab}	28,5 ± 0,3 ^{ab}	3,3 ± 0,1 ^a	1,6 ± 0,1 ^{ab}	61,8 ± 0,5 ^{de}	18,8 ± 3,2 ^{ab}
F3	4,9 ± 0,2 ^{ab}	28,6 ± 0,2 ^{ab}	3,3 ± 0,0 ^a	1,6 ± 0,0 ^{ab}	61,6 ± 0,4 ^{cde}	13,8 ± 2,3 ^a
F4	4,9 ± 0,2 ^{ab}	28,4 ± 0,1 ^{ab}	3,5 ± 0,0 ^{bc}	1,6 ± 0,0 ^{ab}	61,5 ± 0,1 ^{cde}	30,3 ± 0,5 ^d
F5	4,9 ± 0,1 ^{ab}	28,8 ± 0,3 ^{abc}	3,5 ± 0,0 ^{bc}	1,8 ± 0,2 ^b	61,0 ± 0,4 ^{bc}	24,9 ± 2,0 ^c
F6	4,9 ± 0,0 ^{ab}	29,1 ± 0,9 ^{bc}	3,5 ± 0,1 ^{bc}	1,9 ± 0,0 ^c	60,6 ± 0,9 ^b	17,9 ± 2,3 ^{ab}
F7	5,0 ± 0,1 ^b	28,1 ± 0,2 ^a	3,5 ± 0,1 ^{bc}	2,1 ± 0,0 ^{cd}	61,2 ± 0,2 ^{bcd}	38,3 ± 0,5 ^e
F8	5,0 ± 0,2 ^{ab}	28,7 ± 0,3 ^{ab}	3,5 ± 0,0 ^{bc}	2,2 ± 0,0 ^{cd}	60,6 ± 0,1 ^b	35,1 ± 0,7 ^{de}
F9	4,9 ± 0,1 ^{ab}	29,4 ± 0,3 ^c	3,6 ± 0,0 ^c	2,2 ± 0,0 ^d	59,8 ± 0,3 ^a	35,0 ± 5,9 ^{de}
Rata-rata	4,9 ± 0,1	28,6 ± 0,5	3,4 ± 0,1	1,8 ± 0,2	61,2 ± 0,8	27,1 ± 8,8
Referensi	Max. 5%*	Min. 9,5%*	Min. 5%*	Max. 1,6%*	Min. 70%*	22-50 N**

Note: Numbers followed by the same superscript letter indicate no significant difference;* Source (Nasional, 2011); ** Source (Nindiyarani, Sutardi, & Suparmo, 2011).

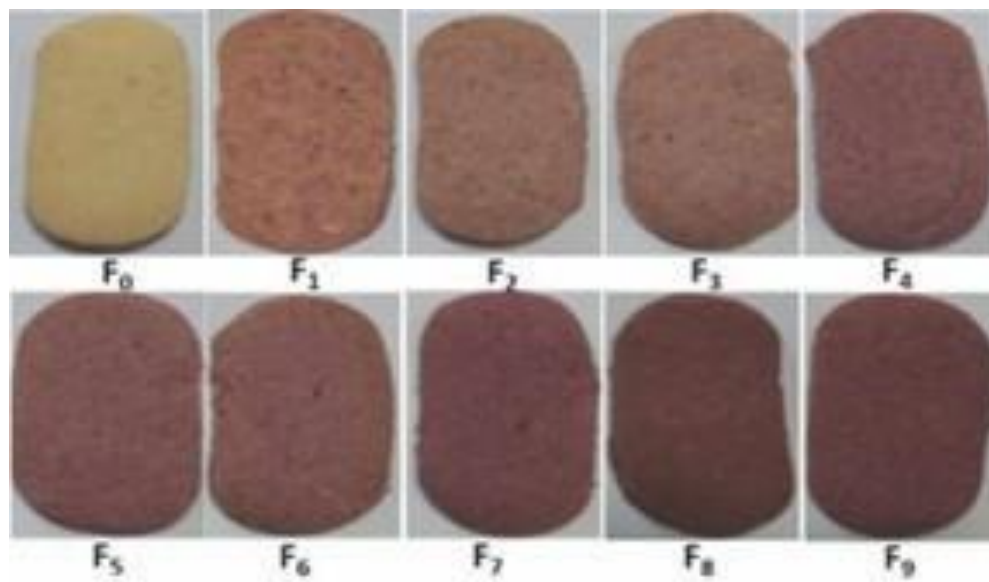


Figure 4. Cookies based on Mocaf flour, purple yam flour and cinnamon powder

Water content

Water content indicates the proportion of water composition in a food ingredient. Based on **Table 3**, the F₇ shows the highest level of water content which was obtained at around 5.0%, while the F₀ shows the lowest level of water content which was around 4.8%. The high

or low water content is caused by the ability of raw materials to absorb the water. Mocaf and purple yam flour have a greater water absorption capacity than wheat flour (Etudaiye, Oti, Aniedu, & Omodamiro, 2015). The water absorption capacity of Mocaf flour and purple yam were around 250 – 300% (Olatunde, Henshaw, Idowu,

& Tomlins, 2016), while wheat flour is around 50 – 60% (Feri Kusnandar, Danniswara, & Sutriyono, 2022). One of the important factors that affect the absorption of water in food is the protein content. The side chain polar groups of protein compounds such as carbonyl, hydroxyl, amino, carboxyl, and sulfhydryl are hydrophilic components, so they can hydrogen bond with water (Rauf & Sarbini, 2015). As shown in **Figure 5**, the water content tends to increase with increasing protein content in cookies. However, in general, the water content in each formulation was not significantly different ($p>0.05$).

Reference to the quality requirements for cookies based on SNI 01-2973-2011 where the maximum water content is around 5% (Badan Standarisasi Nasional, 2011). The water content for all formulations meet the specified criteria where the moisture content of cookies was in the range of 4.8-5.0%.

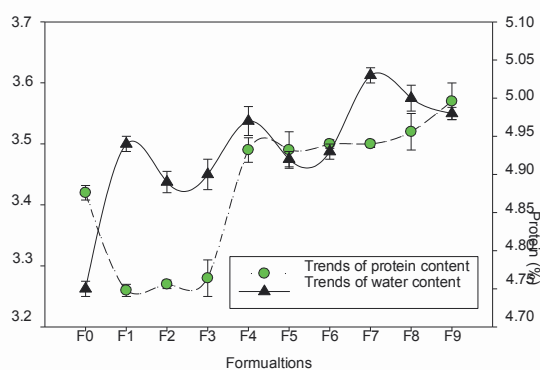


Figure 5. Correlation between protein and water contents

Fat content

Based on **Table 3**, the fat contents were obtained in the range of 28.1 - 29.4%. These fat contents were much higher than the standard set by SNI 01-2973-2011 where the minimum cookie fat content is around 9.5% (Nasional, 2011). These facts show the resulting cookies meet the standards set.

Overall, the fat content in each formulation was not significantly different ($p>0.05$). This fact might be due to the fat content in Mocaf flour and purple yam flour being quite the same, which was in the range of 0.4 – 0.8 g per 100 g, consequently, the changes in composition do

not have a significant effect on the fat content of cookies. In addition to the fat content in Mocaf and purple yam flour, the cinnamon composition also affects the fat content of cookies. According to Singh, Maurya, Delampasona, and Can (2007) that cinnamon contains essential oils which can be categorized as fats (Mulyani & Sujarwanta, 2018). In general, although not significant, the increase in the composition of purple yam flour and cinnamon powder tends to increase the fat content of cookies. The phenomenon of the effect of adding purple yam flour and cinnamon powder on the increase in fat content was shown in **Figure 6**. The higher composition of purple yam flour and cinnamon powder tends to increase the fat content in the cookies.

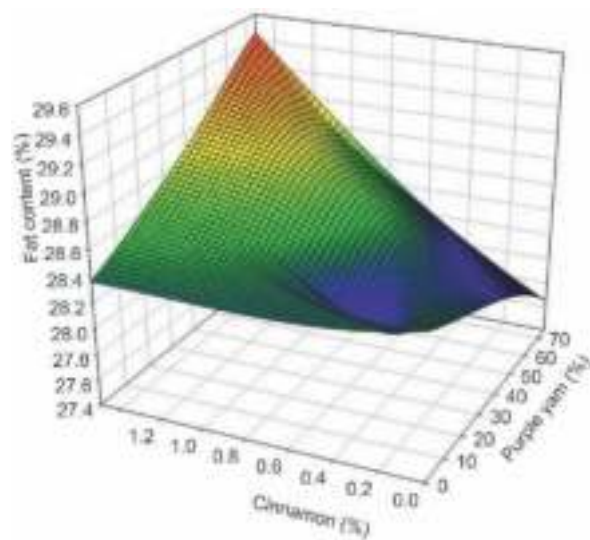


Figure 6. Correlation between purple yam flour and cinnamon powder compositions on fat content in cookies

Protein Total Content

Protein is one of macronutrients that is the most important for the body. Protein content can help repair muscles and create a feeling of fullness. However, consuming cookies that are rich in protein must be limited to prevent a negative impact on health. Based on **Table 3**, the protein contents were obtained in the range of 3.3 - 3.6%. These protein contents were lower than the standard set in SNI 01-2973-2011 which is 5%. The low protein content may be caused by the low protein content in Mocaf, purple yam flour, and cinnamon powder. The highest protein content was found in F₉, while the lowest in F₁. Based

on **Figure 7**, protein content tends to increase with increasing composition of purple yam flour and cinnamon. An increase in the composition of purple yam flour and cinnamon powder had a significant effect on protein content ($p < 0.05$). This means the higher the composition of purple yam flour and cinnamon powder, so the higher the protein content of cookies. This might be due to the protein content in purple yam flour (3.8%) and cinnamon (2.5%). As described above, the F_9 formulation uses the most purple yam flour and cinnamon powder, so the protein content was the highest (3.6%).

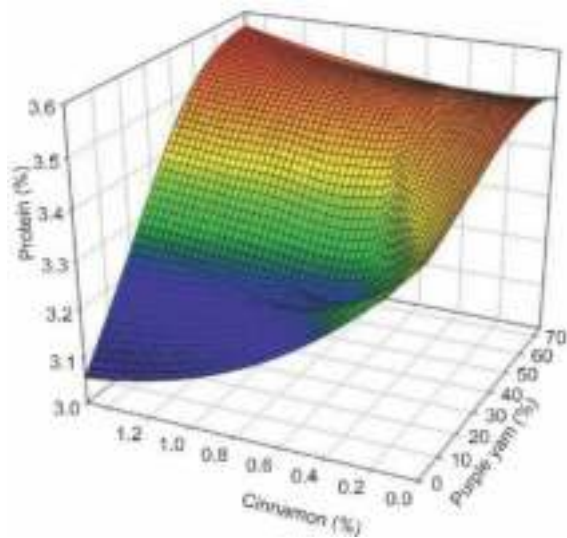


Figure 7. Effect of purple yam flour and cinnamon powder compositions on the changes in protein content

Ash content

Although the ash content does not have a direct effect on health, it needs to analyze to find out a general description of the mineral content in food products. The ash content in food refers to the minerals that remain after all the organic compounds have been burned during the ashing process. Table 3 shows the ash content of cookies, where the highest content was around 2.2% (F_9), while the lowest was around 1.5% (F_0). These results illustrated that the ash content was affected by the high composition of purple yam flour and cinnamon powder. Based on the preliminary test, it was known that the ash content in purple yam was around 1.8%. So that the highest formulation of purple yam flour will have the highest ash content.

In addition, the use of cinnamon powder also had a significant effect ($p < 0.05$) on the ash content. It was well known that cinnamon powder contains the highest ash compared to other ingredients, which was around 4.0%. This fact was caused by cinnamon powder containing calcium oxalate, glycyrrhizin, asparagine, essential oils, and other components (Herawati et al., 2018). As shown above, F_0 has the lowest ash content because Mocaf flour contains the lowest ash content compared to other ingredients, which was 0.4%. The correlation between the composition of purple yam potato flour and cinnamon powder on ash content were shown in **Figure 8**.

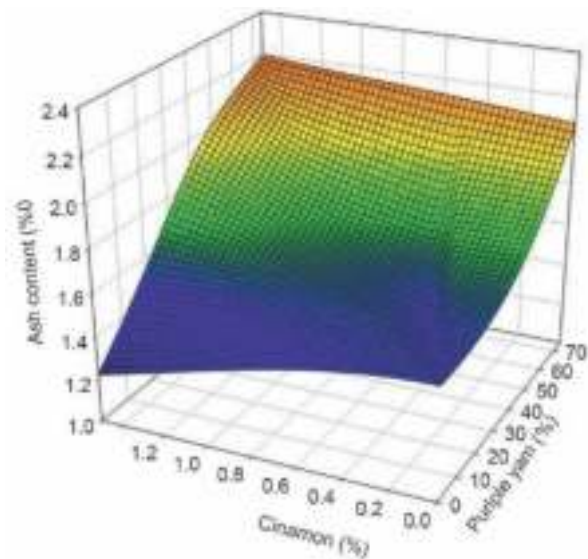


Figure 8. Effect of purple yam flour and cinnamon powder compositions on ash content

The standard of the quality requirements of cookies based on ash content has been stipulated in SNI 01-2973-2011, where the maximum ash content of cookies is 1.6% (Nasional, 2011). The results of the analysis showed that the formulations of F_0 (1.5%), F_1 (1.6%), F_2 (1.6%), and F_3 (1.6%) met the quality requirements for ash content which were obtained around 1.6%. Meanwhile, the formulations of F_4 , F_5 , F_6 , F_7 , F_8 , and F_9 were not meet with the standard ash content ($> 1.6\%$) set.

Carbohydrate content

Based on the quality requirements of cookies according to SNI 01-2973-2011 that the minimum carbohydrate content that must be fulfilled by cookies is around 70% (Nasional, 2011). As

shown in **Table 3** that the carbohydrate contents of cookies were lower than the standard set where the carbohydrate content was in the range of 59.8 - 62.1%. The low carbohydrate content in cookies might be due to the low carbohydrate content in the raw materials of both Mocaf flour and purple yam flour.

In the context of carbohydrate content, Mocaf flour has undergone quality improvements (Kurniati, Aida, Gunawan, & Widjaja, 2012). During the fermentation process, the starch from cassava flour can be hydrolyzed into maltose and then converted into sugar, as a result, Mocaf flour contains higher carbohydrates (Yani & Akbar, 2018). Therefore, the high composition of Mocaf flour tends to increase the carbohydrate content of cookies. The correlation between the composition of Mocaf flour and purple yam on carbohydrate content was shown in **Figure 9**.

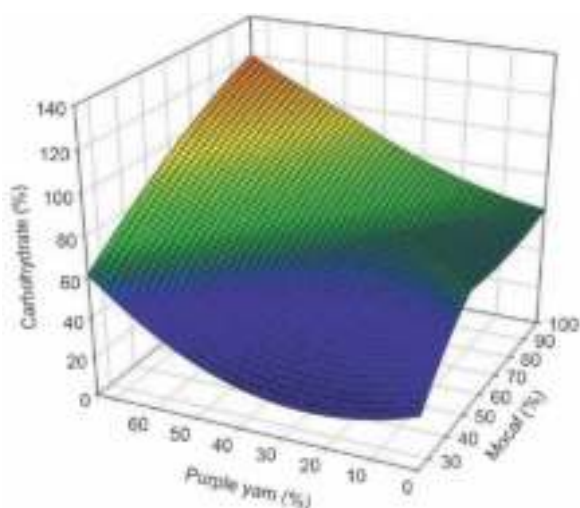


Figure 9. Effect of Mocaf flour and purple yam composition on carbohydrate content in cookies

Hardness

Texture such as hardness is one of the physical parameters that need to be evaluated because it greatly affects the quality of cookies. Cookies that are too hard will be difficult to chew and can reduce the delicious taste. In addition, textures that are too hard also tend to be more brittle and easily crushed, consequently reducing the shelf life. So far, there is no standard hardness value set for cookies, so generally, the results of texture identification are only used to provide information related to the relationship between the formulation

and the hardness value. As shown in Table 3, the highest hardness value was obtained at around 38.3 N (F₇), while the lowest hardness value was obtained at around 13.8 N (F₃). The hardness of cookies was generally affected by water, fat, carbohydrate, and protein contents.

According to Istinganah, Rauf, and Widyaningsih (2017) that starch content (especially amylose) was very associated with the level of hardness. Flour with a high amylose composition tends to produce cookies that were much harder than flour with a low amylose content. Purple yam flour contains about 74.6% starch with 24.8% amylose (Nindyarani et al., 2011) which is higher than the starch content in Mocaf flour which is around 63.1% with an amylose content of around 11.1% (Yani & Akbar, 2018). Therefore, the high composition of purple yam flour tends to increase the hardness level. The phenomenon of the purple yam effect on the level of hardness was shown in **Figure 10**.

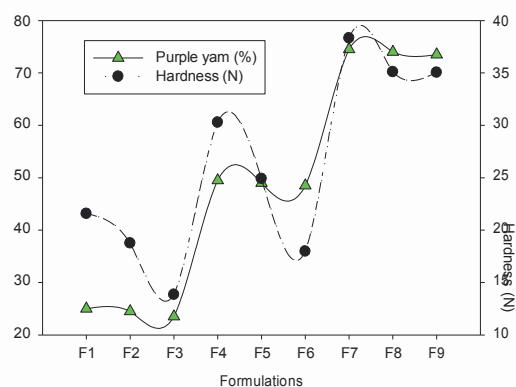


Figure 10. The pattern of increasing the hardness value of cookies was based on increasing the composition of purple yam flour

Antioxidant Activities

The high value of antioxidant activity can describe the functional characteristics of cookies. It is well known that high antioxidant activity has a high ability to counteract free radicals. Antioxidant compounds can prevent the occurrence of an oxidation reaction by preventing the formation of radicals.

Table 4 shows the IC₅₀ values for each formulation. The value of antioxidant activity (IC₅₀) of cookies was obtained in the range of 44.5 - 88.8 ppm. The lowest IC₅₀ value (very strong)

was shown by F₉, while the highest (strong) was shown by F₁. This fact indicates that increasing the composition of purple yam and cinnamon can increase antioxidant activity. This might be due to the purple yam and cinnamon contain various antioxidant compounds. Purple yam contains anthocyanins, β-carotene, vitamin C, and vitamin E which act as antioxidants. Meanwhile, cinnamon contains polyphenols, flavonoids, and caffeine which also act as antioxidant compounds.

The antioxidant activity in purple yam flour was about 83.7% with anthocyanin levels were around 391.1 mg GAE/100 g (Tamaroh & Sudrajat, 2021). Meanwhile, cinnamon contains antioxidant compounds, especially cinnamtannins B1 and B2, oligomeric procyanidins, and proanthocyanidins. The antioxidant activity (%RSA) of cinnamon powder was around 90.0% with an antioxidant level of around 355.0 mg GAE/100 g (Shahid et al., 2018).

According to the research results of Fitriani *et al.* (2019) regarding the antioxidant activity in cookies using 80% purple yam flour (without cinnamon powder) showed the IC₅₀ value was obtained around 75.3 ppm (strong antioxidant activity). Whereas, in this study, the F₇ cookies with a purple yam flour composition of 74.5% show a much lower IC₅₀ value (stronger activity), which was around 50.6 ppm. This fact indicated that the addition of cinnamon powder contributed significantly ($p < 0.05$) to the increase in antioxidant capacity.

Table 4. Antioxidant activities of cookies

Sample	IC ₅₀ (ppm)	Categories
F ₀	88,7 ± 0,6 ^g	Strong
F ₁	88,8 ± 0,5 ^g	Strong
F ₂	80,2 ± 0,3 ^f	Strong
F ₃	74,9 ± 1,3 ^e	Strong
F ₄	67,3 ± 0,8 ^d	Strong
F ₅	66,0 ± 1,5 ^d	Strong
F ₆	55,7 ± 1,7 ^c	Strong
F ₇	50,6 ± 0,2 ^b	Strong
F ₈	49,5 ± 0,8 ^b	Very strong
F ₉	44,5 ± 1,6 ^a	Very strong
Reference	70-8 ppm*	Strong
	62,3 ppm**	Strong

Note: Numbers followed by the same superscript letter indicate no significant difference; * Source (Fitriani et al., 2019); **Source (Hati, Setiani, & Bintoro, 2020)

Sensory Evaluation

The sensory properties of cookies including color, taste, texture, aroma, and overall preference were presented in **Table 5**. Color is a parameter attached to cookies that is first seen (Tarwendah, 2017). Based on the color analysis the color F₇ was the most preferred, where the level of preference was around 3.9 (like criteria). While the color F₀ was the least preferred with a preference level of around 3.1 (neutral category). The appearance of a strong purple color on F₇ makes cookies more attractive to attention than F₀ which was brown (See Figure 1). According to Nabilah *et al.* (2019) which stated that the most preferred color of cookies was cookies with the highest purple sweet potato flour composition, namely 75%.

Taste is one of the sensory parameters of cookies. The taste of the F₉ formulation was the most preferred where the level of preference was observed around 4.1 (like), while the F₀ formulation was least preferred with a level of preference of around 3.4 (neutral). According to Widyasitoesmi (2010) that purple yam has a distinctive taste and tends to be sweet so it was preferred over Mocaf flour which does not have a distinctive or sweet taste (Setyadjid & Setiyaningrum, 2022). In addition to purple yam flour, increasing the composition of cinnamon powder can also improve the taste and flavor of cookies because cinnamon powder contains cinnamaldehyde compounds which act as flavor and flavor formers (Shobur, Hersoelistyorini, & Syadi, 2021). Thus, the high composition of purple yam flour and cinnamon powder (formulation F₉) resulted in a balanced taste that the panelists preferred.

As with color and taste, aroma is also an important parameter in food products. Aroma is a parameter attached to food products that can be identified using the sense of smell. Based on the aroma evaluation that the F₉ formulation was the most preferred with a preference level of around 3.8 ± 0.8 (liking criteria). This might due to the aroma of purple sweet potato flour was not so strong. In addition, the use of cinnamon powder in the composition also makes the aroma more attractive. While the F₀ formulation was the least preferred with a preference level of around 3.2 ± 1.0 (neutral criteria). This might be caused by the

emergence of a sour aroma caused by the high composition of Mocaf flour. Mocaf flour has a distinctive cassava flavor and a slightly sour aroma (Yani & Akbar, 2018), as a result, the higher the Mocaf flour composition causes the stronger the sour aroma in the cookies.

Texture parameters also greatly affect the quality of cookies. The harder or softer the texture of the cookies describes the worse the quality. Texture can be judged by biting, chewing, and touching. Based on the texture analysis, the F₂ was the most preferred cookie with a preference level of around 3.9 ± 0.7 (like criteria), while the F₇ was the least preferred with a preference level of around 3.5 ± 0.9 (neutral criteria). Reducing

the composition of purple yam flour (decreasing amylose content) resulted in a decrease in the level of hardness. Formulations F₂ and F₃ used the purple yam flour at 24% and 23.5%, respectively, so their textures were less hard. The level of preference decreased with increasing the purple yam flour composition because the cookies tended to be harder. According to the result that was reported by (Setyadjid & Setiyaningrum, 2022) that cookies with a low composition of purple yam flour (30%) were the most preferred texture. In addition, the results of Nindyarani et al. (2011) also stated that cookies with a low purple sweet potato flour composition (about 25%) were the most preferred.

Table 5. Sensory of cookies

Sample	Hedonic tests				
	Color	Taste	Texture	Aroma	Overall
F ₀	3,1 ± 1,3 ^a	3,4 ± 1,0 ^a	3,6 ± 1,1 ^a	3,2 ± 1,0 ^a	3,4 ± 1,1 ^a
F ₁	3,4 ± 0,8 ^{ab}	3,6 ± 0,9 ^{ab}	3,7 ± 0,6 ^a	3,5 ± 0,7 ^{ab}	3,8 ± 0,8 ^a
F ₂	3,4 ± 0,9 ^{ab}	3,8 ± 0,9 ^{ab}	3,9 ± 0,7 ^a	3,6 ± 0,7 ^b	3,9 ± 0,7 ^a
F ₃	3,2 ± 0,9 ^a	3,8 ± 1,0 ^{ab}	3,9 ± 0,7 ^a	3,6 ± 0,6 ^{ab}	3,7 ± 1,0 ^a
F ₄	3,8 ± 0,6 ^b	3,9 ± 0,8 ^{ab}	3,7 ± 0,9 ^a	3,7 ± 0,7 ^b	3,8 ± 0,7 ^a
F ₅	3,8 ± 0,7 ^b	3,8 ± 0,9 ^{ab}	3,7 ± 0,9 ^a	3,6 ± 0,7 ^{ab}	3,7 ± 0,8 ^a
F ₆	3,8 ± 0,9 ^b	3,9 ± 0,9 ^{ab}	3,7 ± 0,7 ^a	3,7 ± 0,5 ^b	3,9 ± 0,8 ^a
F ₇	3,9 ± 0,9 ^b	4,0 ± 0,9 ^b	3,5 ± 0,9 ^a	3,6 ± 0,9 ^{ab}	3,6 ± 1,0 ^a
F ₈	3,8 ± 0,9 ^b	3,9 ± 0,9 ^{ab}	3,6 ± 0,9 ^a	3,5 ± 0,9 ^{ab}	3,5 ± 0,9 ^a
F ₉	3,8 ± 1,0 ^b	4,1 ± 0,7 ^b	3,7 ± 0,8 ^a	3,8 ± 0,8 ^b	3,8 ± 0,9 ^a
Average	3,6 ± 0,9	3,8 ± 0,9	3,7 ± 0,8	3,6 ± 0,8	3,7 ± 0,9 ^a
Reference	2,1 - 3,1 [*]	1,8 - 3,6 [*]	4,3 - 5,7 ^{**}	2,3 - 3,0 [*]	3,3 - 4,0 ^{***}

Note: Numbers followed by the same superscript letter indicate no significant difference; *Source (Nabilah, 2019); **Source (Nindyarani et al., 2011); ***Source (Setyadjid & Setiyaningrum, 2022)

CONCLUSION

The physicochemical properties, antioxidant activities, and sensory of cookies made from Mocaf flour and purple yam have been evaluated. The results of the physicochemical properties test showed that the cookies produced could not meet all the established quality standard criteria. However, in terms of antioxidant activity showed that the use of Mocaf flour, purple yam flour, and cinnamon powder can produce cookies with strong and very strong criteria of antioxidant activity. Formulations of F₈ and F₉ show the highest antioxidant activity (IC₅₀), which were around 49.5 ppm and 44.5 ppm, respectively (very strong category). The high composition of purple yam

flour and cinnamon powder tends to provide better physicochemical characteristics and antioxidant activity (IC₅₀) compared to the use of Mocaf flour only. The sensory results showed that F₂ was the most preferred cookie compared to other formulations. Further research is needed to get the right formulation to produce cookies with balanced physicochemical properties, antioxidant activity, and sensory.

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THE ROLE OF MINERAL AND SYNBIOTIC TO ENHANCE IMMUNITY DURING COVID-19 PANDEMIC : A LITERATURE REVIEW

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ABSTRACT

COVID-19 has become a pandemic in the last 3 years worldwide and cases cause high mortality and morbidity. To reduce COVID-19 infection, we need to keep our immune system healthy. Several nutrients have been shown to have specific abilities to increase the power of the immune system, but their use in the treatment of COVID-19 is still being debated. This review aims to determine the role of minerals and synbiotics in increasing immunity during the COVID-19 pandemic. Specific minerals such as zinc, selenium, iron and copper have promising potential to treat COVID-19 by reducing clinical impact, markers of inflammation, and improving immunological biomarkers. In addition to increasing mineral intake, maintaining a healthy immune system can also be done by improving the health of the gut microbiota. One of the therapies that is considered to have a positive impact on handling COVID-19 is using synbiotics (a combination of prebiotics and probiotics). However, the safety and efficacy of mineral and synbiotic supplementation in COVID-19 patients as adjunctive therapy still requires further research. Minerals and synbiotics can help boost the immune system and reduce symptoms during a COVID-19 infection.

Keywords: COVID-19; immunity; mineral; synbiotic; SARS-CoV-2

INTRODUCTION

Coronavirus Disease-19 (COVID-19) is an acute infectious respiratory disease transmitted through droplets and caused by an RNA virus called SARS-CoV-2. World Health Organization has announced that COVID-19 are globally pandemic after its outbreak in all countries. Indonesia itself has gone through third wave of COVID-19 cases with more than 6 million positive cases and 157 thousand death cases on 26 May 2022. COVID-19 specifically gives symptoms like fever, headache, difficulty of breathing, dyspnea, dry cough, vomit, diarrhea, and for several cases leave invasive lesion in the lungs (C. Huang et al., 2020; Shi et al., 2020). Severity of the COVID-19 cases are very diverse and depends on the occurrence of several comorbidities like cardiovascular disease, hypertension, metabolic syndrome, lung diseases, and diabetes mellitus type 2. The mortality also higher in older people and has at least one comorbidities (Mungroo et al., 2020; Zheng et al., 2020).

As it rapid transmission and evolution, until now there are no specific drugs that have been found to cure or prevent COVID-19 infection. So that it is important for people to always make sure their immune system in its best condition so that can fight COVID-19 without further symptom. Human immunity is a complex system that need several nutrients in order to make immune cells work optimally and can combat pathogenic agents (Shetty, 2010; Wood, 2006). One of which mineral that plays major role in providing better immune system, namely zinc, iron, selenium, and cooper. Several studies has showed information about the correlation between mineral and immune system both innate and adaptive (Gombart et al., 2020; Wintergerst et al., 2007). Deficiencies of several mineral known can affect immune cell function and make people more susceptible to infections (Calder et al., 2020; Gombart et al., 2020). So that it is important to make sure adequate intake of mineral during COVID-19 pandemic.

In order to gain an optimal immune system against COVID-19, it is necessary to also maintain

gut health. It is important because the SARS-CoV 2 can enter bloodstream through Angiotensin Converting Enzyme (ACE2) receptor which mostly can be found in digestive tract (Li et al., 2003; Zou et al., 2020). This also answer why many cases of COVID-19 has developed digestive symptom during infection period. Several studies has shown that the usage of synbiotic (combination of prebiotic and probiotic) can act as prevention to infections related to gut by helping balance intestinal microecology, improve the microbiota dysbiosis, and prevent secondary infections caused by bacterial translocation (Xu et al., 2020). But, the connection between synbiotic and COVID-19 still unclear and need further studies.

Reviewing the needs to explore the connection between mineral and synbiotic intake, it is necessary to make a review to investigate these correlation. In this review, we assessed the role of mineral and synbiotic in supporting the immune system and its correlation to COVID-19.

THE ROLE OF MINERAL IN THE IMMUNE SYSTEM

1. Zinc

Zinc takes significant part within the immune system. The free form of zinc has an immediate antiviral effect (Alpert, 2017). The daily requirement for zinc is 8-11 mg/day, with an upper limit of 40 mg/day. Zinc intake as many as 30-50 mg/day during infection is recommended to control RNA virus, such as influenza and coronavirus (Institute of Medicine, 2001; McCarty & DiNicolantonio, 2020). If zinc deficiency occurs, then there will be an increase in the risks of viral infection, thymic atrophy, lymphopenia, and decreased lymphocyte responses. Zinc can be found in lean red meat, whole grain cereals, nuts and legumes (Hidayati et al., 2019).

Zinc can inhibit enzymatic activities, SARS-CoV RNA polymerase replication, and inhibit ACE2 activities. Zn^{2+} also reduces the permeability of cell membranes without damaging nor penetrating the cells. Zinc provides protective effects in the prevention and COVID-19 therapy, where zinc increases the capillary epithelial barriers

and inhibit the transcapillary protein plasma movement. Therefore, zinc reduces local oedemic incidents, inflammation, exudation, and mucus secretion, preventing lung injury due to the use of a ventilator, modulating the antiviral immunity, and being a regulator of the tight junction of ZO-1 and Claudin-1 proteins to increase its barrier functions so that the virus can be prevented (Hunter et al., 2020; Skalny et al., 2020).

Zinc is vital for cell growth and differentiation of both innate and humoral immune cells, and also modulate cytokine release and trigger T cell $CD8^+$ proliferation (Wintergerst et al., 2007). Zinc is also vital for the intracellular binding of tyrosine kinase at T cell receptors, which is required for the development and activation of T lymphocytes (Wintergerst et al., 2006). Furthermore, zinc is a cofactor for 750 transcriptional factors for protein synthesis related to the immune and a cofactor for 200 enzymes involved in the formation of antioxidants, such as superoxide dismutase (SOD) and SMAD anti-inflammatory protein, by stabilizing the tertiary structure and being an essential component on the catalytic site of enzymes (Andreini et al., 2011; Gammoh & Rink, 2017). Zinc is needed in the production of the metallothionine antioxidant complex that is responsible for the lungs' elasticity. Moreover, it has been noticed that zinc plays a role in doubled-reducing the mortality rate due to pneumonia in people with adequate zinc intake (Barnett et al., 2010).

Zinc supplementation causes transient zinc chelation by N,N,N',N'-tetrakis(2-pyridinylmethyl)-1,2-ethanediamine (TPEN) to induct the antiviral inside cells through the activation of NF- κ B that triggers the interferon signaling. Zinc also roles as an anti-inflammatory agent, which triggers the development of Treg, Th17, and Th9 cells and helps the production of IgG antibody (Bonaventura et al., 2015; Gombart et al., 2020; Subramanian Vignesh & Deepe Jr, 2016). Zinc is a part of some antiviral compounds, namely zinc N-ethyl-N-phenyldithiocarbamate (EPDTC). Zn^{2+} ion also triggers viricidal activities by damaging the receptors on the surface of the viral cell through ions-centered tetrahedral geometric coordination that functions as an inhibitor against 3C and 3C-like proteases

(Lee et al., 2009). Zinc is also able to decrease the expressions of IL-6 plasma, IFN- α , IL-1b, and TNF- α genes. On the other hand, zinc can increase the IFN- α mediated by JAK1/STAT1 through signaling and increase antiviral enzyme, for instance, latent ribonuclease (RNase L) and protein kinase RNA (PKR), which results in RNA degradation and RNA translation inhibition (Günzel & Yu, 2013).

In vitro study revealed that zinc could reduce the ability of RNA replication by inhibiting RNA polymerase as in coronavirus (Martindale et al., 2020). The antiviral zinc-finger protein complex (ZAP) controls the process of virus entry, DNA/RNA replication, and the spread of viral infections (Wang et al., 2010). ZAP ACCHC3 can bind to RNA and facilitate intracellular RNA detection by activating retinoic acid-inducible gene-I (RIG-1)-like receptors (RLRs) and MDA5. The process then causes the kinases such as TBK1 and I κ B phosphorylates the interferon regulatory transcription factor 3 (IRF3) and I κ B- α (inhibitor of NK- κ B) that increases the type-1 interferon. IFN- α triggers the signal to escalate the antiviral protein (RNase L and PKR) that degrades and restrains the process of RNA translation. Zinc inhibit the NK- κ B activities using A20 (ZAP) protein expression that decreases TNF receptor regulation and initiates TLR-NK- κ B tracks. Zinc also acts as a cyclic nucleotide phosphodiesterase (PDE). When PDE is inhibited, it will increase cyclic nucleotide guanosin monophosphate (cGMP) which activates PKA (protein kinase A) and inhibits NK- κ B.

2. Iron

Iron is one of the essential nutrients for the body with various functions, including energy metabolism, growth and development, and the immune system (Sundari & Nuryanto, 2016). Iron can be found in numerous food sources, for instance, red meat, liver (beef and chicken), beans, red rice, and dark green leafy vegetables (spinach, kale, and others) (Calder, 2020). Iron is needed by the ribonucleotide reductase enzymes to synthesize the DNA, which functions to form lymphocyte-T cells. Iron deficiency can impaired the myeloperoxidase enzyme functions in the immune system (Sundari & Nuryanto, 2016).

Care needs to be taken in providing iron supplementation in people suffering from infectious diseases. Studies in tropical areas affirmed that iron administration to children with a dose above a certain threshold could escalate the risk of malaria and other infections, including pneumonia. Therefore, the intervention of iron in malaria-endemic areas is not recommended due to several reasons. First, excess iron may lead to the disruption of the immune functions. Second, excess iron can worsen the inflammation. Third, microorganisms need iron to support the growth of the pathogen (Cherayil, 2010; Drakesmith & Prentice, 2012; Ganz, 2018; Ganz & Nemeth, 2015; Nairz et al., 2017, 2018; Oppenheimer, 2001; Weiss, 2002).

Based on those reasons, hence, some methods have been developed to restrain iron-binding or used by pathogens. A study revealed that the provision of iron as much as 50 mg for four days in a week to school-aged children with iron deficiency increased the risk in respiratory tract infections. On the other hand, the addition of omega-3 PUFA as much as 500 mg for four days a week can decrease the adverse effects of iron supplementation (Malan et al., 2015). A meta-analysis study in Chinese children disclosed that those who undergo recurrent respiratory tract infections tend to be lack of iron on their hair (Mao et al., 2014). Thus, it can be implied that the administration of iron must be precise, whether the doses, the patient's condition, or the way of administering.

3. Selenium

Selenium was discovered by John Jakob Berzelius, a Swedish scientist, in 1817. According to Avery J.C. and Hoffman PR, selenium in the human immune system can be studied from the perspectives of immunobiology, leucocyte function increase, and the immune response towards pathogens and anti-cancers (Avery & Hoffmann, 2018). In general, seafood and internal organs are rich sources of selenium. In addition, meat, whole grains, dairy products, and eggs are also good sources of selenium (Kusmana, 2017).

Selenium deficiency can generate immune-incompetence, which will enlarge the risks of viral infections. Epidemiological study in China revealed the positive correlations between the

population selenium levels and COVID-19 recovery rates in 17 cities (Zhang et al., 2020); the higher the selenium level in the body, the faster the recovery of COVID-19 patients. Selenium is one of the micronutrients with essential roles in the immune system, particularly in suppressing the occurrence of oxidative stress. COVID-19 includes in viral infections related to the increase in oxidative stress by enhancing enzyme-producing ROS. Selenium, in the form of sodium selenite reduces the ROS production and the apoptosis of infected cells (Kretz-Remy & Arrigo, 2001).

RNA virus could be a trigger of NF- κ B (Nuclear Factor kappa B) activation. The activation of NF- κ B in cells infected with the nucleocapsid protein from the SARS-CoV can cause the severity of inflammation in lung lesions in SARS patients (Liao et al., 2005). Selenium has a role as the NF- κ B inhibitor among mice exposed to the SARS-CoV, which relates to the survival/immunity (DeDiego et al., 2014).

Besides the functions that have been elucidated, selenium also enhance the activity of the glutathione peroxidase (GSH-Px) (Ghneim, 2017). Selenium in the glutathione peroxidase acts as the catalysator in breaking down the peroxides to be a non-toxic/non-reactive bond. Together with vitamin E, selenium can protect endothelial cells/cell membranes that become the target of SARS-CoV-2 infection (Brigelius-Flohé et al., 2003). The integrity of cell membranes is fundamental, given the cytokine production is determined by the receptor in the cell membrane; hence, selenium is influential in increasing cellular immunity. Selenium is also an antioxidant that boosting the immune system. Selenium deficiency has a significant impact on the activity of selenoprotein antioxidant (specifically Gpx 1 expression) and on reducing the mRNA signal related to the inflammatory pathways. Thus, reducing the body's resistance against the viruses (Z. Huang et al., 2012).

4. Copper

Copper acts as the cofactor in the cellular metabolic reactions and copper-dependent enzymes catalyst reactions that involve molecular oxygen species. Several copper enzymes play a role in the

body's antioxidant defenses (Shetty, 2010). Copper is a micronutrient needed by pathogens and the host during the viral infection. Copper support Th cells, B cells, neutrophil, NK cells, and macrophage that influences the innate and adaptive immune responses (Raha et al., 2020). Copper also supports macrophage functions (copper accumulates in the phagolysosomes of macrophages to fight infectious agents), neutrophil, monocytes, and also increases the activity of NK cells. Furthermore, copper plays a role in the differentiation and proliferation of T cells, as a component of intrinsic antimicrobial which has anti-inflammatory action, antioxidant, and oxidative burst (Gombart et al., 2020). It is believed that copper has a role in the inflammatory responses given copper is a part of Cu/Zn SOD enzymes, which are the keys in the defense against ROS in maintaining the balance of intracellular antioxidant along with selenium and zinc (Gombart et al., 2020; Wintergerst et al., 2007).

The data regarding copper deficiency in humans is very limited due to lack of efficiency usages, homeostasis, and the appropriate parameters to determine the status of copper. The sufficient amount of copper intake enhanced the Th1 responses, decrease T cell proliferation, and increase B cell circulations. A high dose of copper intake (7 mg per day) for healthy adult males in an extended period can reduce the percentage of neutrophilic circulation, IL-2 serum receptor, and antibody titers against influenza virus strain Beijing. On the contrary, the same dose for the same subjects can increase the average immune responses (IL-6). Moreover, there is a pro-oxidant effect that makes this high dose of intake protect red blood cells against peroxidation induced in vitro (Wintergerst et al., 2007).

Copper can kill certain contagious viruses, such as bronchitis virus, poliovirus, HIV type 1, both enveloped and nonenveloped viruses, and single or double-stranded DNA and RNA viruses. Thus, the addition of copper intake can encourage both the innate and adaptive immune systems (Raha et al., 2020). However, until recently, the registered trials to disclose the impact of copper supplementation on COVID-19 patients is not yet published.

THE ROLE OF SYNBIOTIC IN THE IMMUNE SYSTEM

The use of synbiotic (combination of prebiotic and probiotic) in preventing the risks of infections began to be noticed. The Zhejiang Hospital of China recommended the provision of synbiotic in COVID-19 patients to help balance intestinal microecology, improve the microbiota dysbiosis, and prevent secondary infections caused by bacterial translocation (Xu et al., 2020).

1. Probiotics

Some studies have revealed the effects of probiotics (*Bifidobacterium* and *Lactobacillus*) provisions in reducing respiratory infections (de Araujo et al., 2015; Ichinohe et al., 2011). Probiotics can escalate the interferon and the number and activities of antigen, NK cells, T cells, as well as specific antibody both systemic and mucosal (Namba et al., 2010; Zelaya et al., 2016). Probiotics are proven influential in regulating pro-inflammatory and immunoregulatory cytokines that control the clearance virus and prevents lung damages caused by the immune responses. *Lactobacillus plantarum* DR7 is affirmed to be able to suppress the proinflammatory plasma cytokines (IFN-gamma and TNF-alpha), increasing the anti-inflammatory cytokines (IL-4, IL-10), and decreasing the plasma peroxides and the oxidative stress (Chong et al., 2019). It is important in COVID-19 patients which experiencing the cytokine storm. Probiotics can also enhance the tight junction integrity and production of the short chain fatty acid (SCFA) Butyrate, and provide nutrition for colonocytes thus, reduce the SARS-CoV-2 invasion (Baud et al., 2020). Studies also found that probiotics could upsurge the amount of leucocyte, neutrophil, IL-2, TNF-beta, decrease the cytokine expressions (TNF-alpha, IL-1beta, IL-6, IL-8, IL-5, IL13), and IgA saliva level can produce bacteriocin and reuterin, promote phagocytosis, and can maintain Th1 and Th2 homeostasis (Fooks & Gibson, 2002; Guillemard et al., 2010).

Lactobacillus plantarum, as one type of probiotics has been shown to have antiviral activities against coronavirus in the intestinal epithelial cells. *L.plantarum* can also provide IFN- λ 3 to suppress the enteric coronavirus infection and can be used as an alternative antiviral therapy

(Liu et al., 2020). Several meta-analyses showed the presence of probiotic effects (*Lactobacillus rhamnosus* GG, *Bacillus subtilis*, and *Enterococcus faecalis*) in decreasing the incidence and the viral infection duration of the critically ill patients with respiratory tract infections (Hao et al., 2015; King et al., 2014). Xu et al., in their study, concluded that many COVID-19 patients in China experience dysbiosis of intestinal microbiota, which is marked by the decrease in *Lactobacillus* and *Bifidobacterium* because the use of antibiotics and COVID-19 causes diarrhea (Xu et al., 2020).

Probiotics, such as *Lactobacillus plantarum*, *Lactobacillus casei*, *Bifidobacterium animalis*, *Bacillus coagulans*, *Streptococcus salivarius*, and *Enterococcus faecium* have proinflammatory interleukin inhibitor effects. On the other hand, *Lactobacillus gasseri*, *Lactobacillus rhamnosus*, and *Bifidobacterium longum* are acknowledged for their ability to increase the antibody. *Bifidobacterium animalis* can prevent the coronavirus replication by lowering the inositol-requiring enzyme 1 (IRE1) pathway, thereby reducing interleukin 17 (Bozkurt et al., 2019). *Lactococcus lactis* JCM5805 activates plasmacytoid dendritic cells (pDC), where the pDC acts as the cells that produce IFN1 (Siegal et al., 1999; Trinchieri & Santoli, 1978) and mucosal T cells (Tezuka et al., 2011). Moreover, pDC can directly prevent viral spread and replication (Theofilopoulos et al., 2004), and activate the NK cells (Tezuka et al., 2011). Additionally, some probiotics, for instance, *Enterococcus faecium* HDRsEf1, can reduce the mRNA TLR4, TLR5, TLR7, and TLR8 (Tian et al., 2016).

Probiotics in Indonesian foods can be found from sayur asin, tempoyak, mandai, tape, growol tempe, kecap, bakasang, dadih, and many more. Mostly these foods are rich in lactic acid bacteria that good to our health (Nuraida, 2015).

2. Prebiotics

Prebiotics, which are undigested carbohydrates such as inulin, polydextrose, oligosaccharides, fiber, and resistant starch, are used by intestinal microbes for fermentation. Prebiotics are also acknowledged to increase the immunity and the diversity of the gut microbiota, as well as aiding digestion (Bouhnik et al., 2007). As an example,

prebiotics obtained from wheat is proven to reduce the proinflammatory cytokine IL-6 and to boost the anti-inflammatory cytokine IL-10 (Keim & Martin, 2014; West et al., 2017). Prebiotics such as wheat bran, fructooligosaccharides (FOS), and galactosaccharides (GOS) can increase the butyrate levels that reduce inflammation and improve the respiratory fibrosis (Anand & Mande, 2018). SCFA from prebiotic metabolism strengthens the gastrointestinal association with lymphoid tissue (FALT) (Schley & Field, 2002). Hence, administering prebiotics and probiotics to COVID-19 patients can help to improve the intestinal dysbiosis conditions, thereby accelerating the healing process. Prebiotics can help fight respiratory infections as proven by Trompette et al. in their research, where the subject mice fed with prebiotic dietary fiber experienced an increase in macrophage and a reduction in the production of chemokine CXCL1, which causes neutrophil increases in the lungs, as well as adding the CD8⁺ cell functions (Trompette et al., 2018).

An RCT involving 94 premature babies revealed that the intervention of mixed prebiotic galactooligosaccharide and polydextrose (1:1) or probiotic *Lactobacillus rhamnosus GG* reduces the incidence of respiratory tract infections by 2-30 times compared to placebo (Guillemard et al., 2010). Additionally, the administrations of synbiotic *Pediococcus pentosaceus* 5-33:3, *Leuconostoc mesenteroides* 32-77:1, *L. paracasei ssp. paracasei* 19, *L. plantarum* 2,362 in conjunction with inulin, oat bran, pectin, and resistant starch in critically ill patients with a ventilator are proven to decrease the rates of infections, sepsis, SIRS, length of treatment, the period of using a ventilator, and mortality (Kotzampassi et al., 2006).

In Indonesia, prebiotic are mostly can be found in tuber crops, like gembili, yam, dahlia root, potato, sweet potato, and cassava. Prebiotic also can be found in chicory, artichoke, and garlic (Zubaidah & Akhadiana, 2013).

CONCLUSION

Enhancing immune system during COVID-19 pandemic is necessary. The use of zinc during COVID-19 infection can give better result of treatment. Minerals with anti-inflammatory

and antioxidant properties can help to reduce inflammatory response during COVID-19 infection. The usage of synbiotic also can help enhance immune system by balancing intestinal microecology and microbiota balance so it can help preventing the infection of COVID-19. However, the safety and efficacy of nutritional supplementation, including minerals and synbiotic as adjunctive therapy for COVID-19 patient needs further studies.

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STUDY OF SATISFACTION TOWARDS THE FOOD SERVICES IN THE PPLP AND SKO ATHLETE DORMITORIES IN INDONESIA

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ABSTRACT

Providing food for athletes is a special institutional arrangement that caters to athletes' specific needs. Athletes have different nutritional requirements than the non-athlete population, as they need more nutrients to compensate for the energy expended during training, competitions, and recovery after matches. Additionally, athletes must focus on fluid intake and sufficient nutrients to support post-training recovery for optimal performance. Therefore, meeting the needs and satisfaction of athletes becomes a crucial goal for athlete food service. Satisfaction can be assessed based on various indicators such as variety of menu and ingredients, suitable portion size, taste, serving time, packaging, and food handler hygiene. This study aims to assess athletes' satisfaction with the food service in athlete dormitories. The research is an observational survey with a cross-sectional approach involving 159 athletes aged 13-19 years from PPLP West Java, PPLP DIY, PPLP NTB, and SKO Cibubur. Data collection took place in May and July 2022. The research instrument used was a customer satisfaction survey questionnaire with a Likert scale ranging from 1 to 4, where one indicates 'very poor' and four indicates 'excellent.' The analysis technique used Pearson correlation in SPSS 16.0 software. The research showed a relationship between menu variety, ingredient variety, suitable portion size, serving time accuracy, food packaging, serving area, food handler hygiene, food presentation, and food taste with athletes' satisfaction level. Serving time accuracy, food packaging, hygiene, cleanliness of the serving area, food handler hygiene, food appearance, and taste correlate strongly with satisfaction.

Keywords: food service, athlete, satisfactory study, athlete dormitory

INTRODUCTION

The provision of meals includes a series of activities ranging from menu planning to food presentation and distribution (Widyastuti et al., 2018; Penggali et al., 2021). Based on the type of institution, meal provision for athletes falls under the category of meal provision in specialized institutions because athletes have different needs compared to the general population and need to consider other aspects, such as training

periodization and caloric requirements based on the type of sport (Widyastuti et al., 2018; Penggali et al., 2021). Athlete meal provision is one of the supportive aspects aimed at providing high-quality food for athletes to support their optimal health, which is crucial for enhancing athletic performance (Penggali et al., 2021).

Customer satisfaction is an assessment that aligns with customer expectations regarding various aspects of food service, including taste, cleanliness, healthiness, and nutritional value

(Widyastuti et al., 2018). Various cooking methods can affect the content of active ingredients and nutritional value of the food, as well as the appearance of the food, which can ultimately affect the acceptance of the food (Gliszczynska-Swig et al., 2006; Miglio et al., 2008). The variety of food ingredients and menu preparations also affects athletes' acceptance and satisfaction with the food served. One study by Afriani (2017) on pencak silat athletes in PPLP Yogyakarta showed that athletes' acceptance and satisfaction with staple foods were lower than animal protein, plant-based protein, vegetables, and fruits (Afriani et al., 2017).

Poor assessment of food quality can affect consumers' food intake. If consumers are not satisfied with the food served, it can lead them not to finish their meals or not consume the food. It affects the quantity and quality of nutrients entering the consumers' bodies. Previous research conducted by Marlenywati (2017) at an integrated Islamic school found that 80% of students had nutrient intake below the Recommended Dietary Allowance for 2013, 70% of students were dissatisfied with the food served because it was not appealing, and 80% were dissatisfied because the food lacked variety (Marlenywati et al., 2017).

Nutrient intake is crucial to support athletes' performance, especially during adolescence when optimal physical growth occurs, requiring an adequate intake of nutrients (Penggalih et al., 2021; Marlenywati et al., 2017). Based on the Nutritional Adequacy Rate in 2019, adolescents aged 15-18 years have a carbohydrate requirement of 292 grams per day, and their calcium, iron, zinc, and magnesium needs are twice as high as other age groups (Permenkes, 2019; Almatier, 2011). These requirements increase further with the additional physical activity burden of athletes. Given this background, a satisfaction study on meal provision at athlete training centres and schools is important.

METHODS

The research in this study is an observational survey with a cross-sectional design. The research was conducted on adolescent athletes aged 13-19 years who trained at the PPLP (Center for Education and Sports Training for Students) in

West Java, DIY, NTB, and SKO (Special School for Athletes) in Cibubur, with a total of 159 subjects. The sampling technique in this study used total sampling by involving all athletes who received food services at the targeted PPLP and SKO institutions and were willing to provide assessments of meal provision. The instrument used was a meal provision satisfaction survey questionnaire conducted in May 2022 at these institutions. The survey questionnaire contained questions related to various aspects of menu variety, types of food ingredients, portion suitability, timeliness, packaging cleanliness, personnel, facilities, and food taste, rated on a Likert scale with scores of 1-4. A score of 1 is considered poor, 2 is considered sufficient, 3 is considered good, and 4 is considered excellent.

After data collection, descriptive analysis tests were conducted to determine the characteristics of the subjects, and Pearson correlation tests were conducted to determine the relationship between each assessment aspect and athlete satisfaction levels. Statistical analysis was performed using SPSS software version 16.0. This research has obtained ethical approval from the Faculty of Public Health and Nursing Ethics Committee numbers KE/FK/0100/EC/2021 with amandemen number was KE/FK/0512/EC.

RESULTS

The age of the research subjects was predominantly in the 13-16 years range, with an average age of 16 years, corresponding to grades X and XI. Regarding gender, more male athletes were participating in the study (Table 1).

Based on testing the relationship between various aspects of satisfaction assessment and the level of athlete satisfaction, the results show that the level of athlete satisfaction is considered sufficient, with an average score of 2.91.

This level of satisfaction is influenced by menu variety, variety of food ingredients, portion suitability for athletes' needs, timeliness of service, presentation packaging, cleanliness and tidiness of the serving area, cleanliness and tidiness of personnel, food appearance, and food taste. The variable with the most robust relationship with the level of satisfaction, as assessed by

Table 1. Characteristic of the participants

Variable	N (%)
Age	
Early adolescent (13-16)	93 (58,4%)
Late adolescent (17-19)	66 (41,6%)
Gender	
Male	81 (50,9%)
Female	78 (49,1%)

Table 2. Relationship between various assessment aspects and satisfaction level

Variable	r	Satisfaction level
		p-value
Punctuality in serving	1**	0,000*
Neat and clean food packaging	1**	0,000*
Clean and orderly presentation area	1**	0,000*
The serving staff maintains cleanliness	1**	0,000*
The appearance of the served food	1**	0,000*
Taste of the food	1**	0,000*
Overall menu variation	0,832**	0,000*
Menu variation of carbohydrate	0,812**	0,000*
Menu variation of animal protein	0,782**	0,000*
Menu variation of plant protein	0,764**	0,000*
Menu variation of vegetable	0,781**	0,000*
Variation of carbohydrate	0,817**	0,000*
Variation of animal protein	0,819**	0,000*
Variation of plant protein	0,837**	0,000*
Variation of vegetable	0,816**	0,000*
Variation of fruit	0,694**	0,000*
The suitability of carbohydrate dish portions to meet the needs	0,851**	0,000*
The suitability of animal-based dish portions to meet the needs	0,825**	0,000*
The suitability of plant-based dish portions to meet the needs	0,877**	0,000*
The suitability of vegetable dish portions to meet the needs	0,781**	0,000*
The suitability of fruit portions to meet the needs	0,805**	0,000*

* significant if $p \leq 0.05$;** there is an association if $r > 0,159$ (r table)

the Pearson correlation test, is the timeliness of service, followed by food packaging, cleanliness and tidiness of the serving area, cleanliness of personnel, food appearance, and food taste (Table 2). The better the service aspects in terms of timeliness of service, neat and clean food packaging, cleanliness and tidiness of the serving area, cleanliness of personnel, and food taste, the higher the level of satisfaction will be.

The assessment of athletes on several aspects of satisfaction in the provision of meals at PPLP DIY, West Java, NTB, and SKO Cibubur is considered satisfactory, with an average score above 2.5 to 3.0 out of a total score of 4.0. This indicates that the majority of athletes have given a positive assessment of the meal provisions at these locations. Therefore, it can be assumed that the meal provisions at these places generally meet the expectations or satisfaction of the athletes in various measured aspects. However, it is still important to continuously monitor and improve the quality of food services to ensure the athletes' satisfaction is consistently maintained.

Regarding menu variety, the average score ranges from 2.7 to 3.0, which falls into the categories of fair to good (Table 3).

Regarding the variety of food ingredient groups processed, the average assessment score ranges from 2.69 to 2.94, which falls into the category of "satisfactory" (Table 4).

Regarding the appropriateness of portion sizes for each dish group, the assessments range from 2.79 to 2.97, which falls into the "satisfactory" category (Table 5).

A good rating with an average score of 3.1 is given to aspects related to timeliness, cleanliness, and the neatness of food packaging, the dining

Table 3. Assessment of menu variation aspect

Variable	N	Mean
		± Std. Deviation
Overall menu variation	159	2,89 ± 0,83
Menu variation of carbohydrate	159	3,03 ± 0,76
Menu variation of animal protein	159	2,94 ± 0,83
Menu variation of plant protein	159	2,79 ± 0,77
Menu variation of vegetable	159	2,7 ± 0,89

Table 4. Assessment of aspects of variations in types of food ingredients

Variable	N	Mean
		± Std. Deviation
Variety of carbohydrate	159	2,94 ± 0,75
Variety of animal protein	159	2,86 ± 0,81
Variety of plant protein	159	2,79 ± 0,76
Variety of vegetable	159	2,69 ± 0,87
Variety of fruit	159	2,86 ± 0,89

Table 5. Assessment of portion suitability aspect

Variable	N	Mean
		± Std. Deviation
The suitability of carbohydrate dish portions to meet the needs	159	2,97 ± 0,72
The suitability of animal-based dish portions to meet the needs	159	2,91 ± 0,72
The suitability of plant-based dish portions to meet the needs	159	2,79 ± 0,79
The suitability of vegetable dish portions to meet the needs	159	2,92 ± 0,81
The suitability of fruit portions to meet the needs	159	2,89 ± 0,89

Table 6. Assessment of aspects related to timeliness, packaging, premises, personnel, presentation, and taste of food

Variable	N	Mean
		± Std. Deviation
Punctuality in serving	159	3,18 ± 0,75
Neat and clean food packaging	159	3,11 ± 0,79
Clean and orderly presentation area	159	3,17 ± 0,73
The serving staff maintains cleanliness	159	3,16 ± 0,77
The appearance of the served food	159	2,83 ± 0,88
Taste of the food	159	2,82 ± 0,85

area, and the staff. Meanwhile, other aspects are considered satisfactory, ranging from 2.82 to 2.83.

DISCUSSION

a. Menu variation

Good food quality enhances athletes' perception of food satisfaction, increasing their attendance in the cafeteria and reducing the likelihood of athletes purchasing food outside the cafeteria (Jamaluddin et al., 2014).

Based on the assessment of 159 athletes, the evaluation of menu variety in the food provision at PPLP NTB, DIY, West Java, and SKO Cibubur tends to be good, with an average score of 2.89 out of a total score of 4 (Table 3). The menu variety that received the highest rating is the carbohydrate menu, with a score of 3.03, followed by the animal protein menu with 2.94, the vegetable menu with 2.79, and the variety of vegetables with 2.7 (Table 3). It can be seen from the reasonably diverse menu options. The carbohydrate menu has options such as white rice, wet rice, yellow rice, fried rice, ulam rice, *daun jeruk rice*, fried vermicelli, fried noodles, and spaghetti bolognese. In the animal protein menu, meat, chicken, eggs, and fish are prepared into various dishes, including hot and sour cook soy sauce, *opor*, *padang* spices, *curry*, *tongseng*, *bali* spices, *rawon*. The vegetable menu has various preparations of tempeh, tofu, and legumes, which are cooked as stir-fry, fritters, dreadlocks, steamed sugar, soup, and fried. The vegetable menu variety consists of stir fry, soup with condiment, soup with coconut milk, stew, sautee.

The data indicates that menu variety, both overall and specific to each menu category (carbohydrates, animal protein, plant-based protein, vegetables), is significantly related to athletes' satisfaction levels in food provision. Research conducted by Baiomy et al. (2017) states that factors influencing consumer satisfaction in food provision include menu descriptions, variety, and design (Jawabreh et al., 2018).

a. Type variation

The variety of food ingredients served (such as carbohydrates, animal protein, plant-based protein, vegetables, and fruits) has been shown to influence athletes' satisfaction levels with food provision significantly. This data aligns with research conducted by Ismail et al. (2019), which stated that with the increasing awareness of consumers in collage cafeteria that there is no single type of food could fulfil all nutritional needs. The availability of various types of

food ingredients (such as fruits, vegetables, carbohydrates, protein, and fats) can attract consumers to dine in the cafeteria (Ismail et al., 2019).

The athletes' assessments of the variety of food ingredients in the food provision show scores from highest to lowest: 2.94 for carbohydrates, 2.86 for animal protein, 2.86 for fruits, 2.79 for plant-based protein, and 2.69 for vegetables (Table 4). Overall, the assessment of the types of food ingredients processed is considered quite good due to the variety in the sources of carbohydrates, including rice, bread, noodles, pasta, potatoes, and flour-based products. The animal protein category combines various ingredients, including chicken, beef, lamb, chicken liver, chicken eggs, duck eggs, freshwater fish, sea fish, squid, shrimp, meat rolls, and fish meatballs. Various types of fruits, such as oranges, snakefruit, melons, watermelons, bananas, and papayas, show good variation. For plant-based protein, the food provision at PPLP and SKO processes various legumes, tempeh, and tofu. Likewise, with vegetables, there are various types, including cabbage, water spinach, spinach, carrots, cauliflower, green beans, long beans, broccoli, corn, green onions, mustard greens, white mustard greens, tomatoes, and bean sprouts.

In PPLP West Java, based on a 6-day cycle, the most frequently appearing type of carbohydrate is white rice with a frequency of 18 times, followed by potatoes 1 time, noodles 1 time, and vermicelli 2 times. The most frequently provided animal protein is chicken, with a frequency of 7 times, followed by fish and eggs 6 times, beef, tofu, and tempeh each 3 times, meat rolls 1 time, and chicken gizzards 1 time. Mixed carrots and cabbage appear most frequently among vegetables, with a frequency of 6 times, followed by green beans, long beans, green mustard, white mustard, red beans, chayote squash, jackfruit, and mushrooms, each appearing once. Fruits are served alternately daily, including melon, banana, orange, papaya, and watermelon.

RAMU	RAMU	RAMU
NASI PUTIH	NASI PUTIH	NASI PUTIH
TONGKOL, SARDEN	KEMAP, SARDEN, PANGKANG	AYAM, KEMAP
TELUR, SPINACH	KEMAS, TELUR	STUP, SPINACH
KEMAPUK	PERKEBES, TEMPE	ORANG, TELUR
TELUR, BUAHAN	ORANGUK	ORANGUK
AIR MINERAL	BUN, BUNAN	BUN, BUNAN
	AIR MINERAL	AIR MINERAL

Figure 1. Example of a one-day menu cycle at one of the PPLP facilities

\ The significant relationship between the variety of food ingredients and customer satisfaction is consistent with research conducted by Tanuwijaya et al. (2019), which showed that the greater the variety of food ingredients used, the higher the level of satisfaction. Furthermore, as the variety of food ingredients increases, the menu options also expand. Consequently, customer satisfaction can increase with a more diverse menu selection.

b. Portion Size

Portion adequacy is one of the crucial aspects of food provision that can influence consumer satisfaction (Agustina, 2016). Standardized portions can be used to predict nutrient adequacy and consumer intake. The amount of food portions athletes need can vary between different types of sports (sports disciplines). For example, endurance sports may require higher calorie intake due to the longer duration of exercise, ranging from 30 minutes to 4 hours, while strength sports generally have shorter exercise durations. Therefore, the quantity and type of carbohydrates needed may differ. In endurance sports, complex carbohydrates are needed in larger quantities than in strength sports, which require carbohydrates in simpler forms (Penggali, 2020). Portion sizes can also vary among individuals based on their preferences, affecting their satisfaction levels (Agustina, 2016). The size of food portions can also impact the visual presentation or appearance of the food, affecting their likability.

The food portions' suitability received a good rating with an average score of 2.79 to 2.97 out of a total score of 4. It indicates that athletes are satisfied with the standardized portions of food served for carbohydrate variants, animal protein, plant-based protein, vegetables, and fruits. This study suggests that portion suitability correlates with athletes' food provision satisfaction. Research on food provision for athletes at PPLP West Java conducted by Putra (2021) also shows a relationship between portion suitability and athlete satisfaction.

Taste, Food Appearance, Presentation Time, Cleanliness of Space, and Service

Providing food specifically for athletes should aim to meet their nutritional needs following the periodization of their training programs (Sedyanti,

2014). Among various assessment aspects, the appearance of food plays a crucial role in athlete satisfaction. Food appearance encompasses several components: presentation, portion size, texture, shape, and colour. The more diverse the food variations across these components, the more attractive it is to consumers (Putra et al., 2021). Additionally, good food appearance must be complemented by delicious taste. Unpleasant taste can diminish food quality and lead to food waste as consumer expectations are unmet (Heikkilä et al., 2016).

In the assessment of food taste, a score of 2.82 out of a total score of 4.0 was obtained (Table 6), indicating that athletes are reasonably satisfied with the taste of the food served. For the aspect of food appearance, the assessment score is 2.83 out of 4.0 (Table 6), indicating a “Satisfactory” level of satisfaction. Meanwhile, for the aspects of serving time, cleanliness of the dining area, and handling of food, scores above 3.0 were obtained, indicating a “Good” rating.

In this study, the accuracy of food serving time is closely related to consumer satisfaction both before and after the nutritionist intervention ($p=0.000$). According to research conducted (Sunarya & Puspita, 2018) on patients at the Sultan Syarif Mohamad Alqadri Hospital in Pontianak, timely meal service can enhance patient acceptability. Timeliness is also important to prevent food waste. Because the timing of meals matches meal times, consumers’ appetites remain intact. The food serving time accuracy also reflects the food service staff’s ability to adjust portion sizes and food composition to match consumers’ meal times (Dewi, 2019). The timing of meals for athletes needs to consider their training schedules. Based on research conducted by (Hasbullah et al., 2017), athletes are typically provided with 3 main meals and 3 snacks, with breakfast and dinner given after athletes finish their training to avoid digestive problems and optimize performance by providing a snack before training. Thus, training can be carried out optimally.

Applying hygiene and sanitation in food provision is essential to prevent food poisoning (Odeyemi et al., 2019). This study shows that consumer satisfaction is influenced by the cleanliness and tidiness of food handlers and

dining areas ($p=0.000$). This is in line with the research conducted by (2007), which found that the cleanliness of food service facilities and food handlers is a significant factor affecting consumers’ decisions to dine at a particular location. Most consumers rely on their judgment to assess hygiene risks, with the cleanliness of food handlers’ hands during food service being the primary assessment frequently conducted by consumers. Additionally, a study by Park et al. (2016) revealed that 5 sanitation dimensions affect consumers’ emotions or feelings towards consuming food at a food service facility: cleanliness of food handlers, dining area, food handling, dining room appearance, and the availability of bathrooms. Therefore, improving the knowledge and behaviour of hygiene in all aspects of food service is an important component, and regular programs should be implemented (Pepple, 2017).

Consumer satisfaction perceptions are also influenced by food packaging. In addition to food storage, packaging also serves as a product representation, maintains the safety and freshness of food, provides nutritional value information, and protects food products during distribution (Claudio, 2012). In the context of food provision at athlete training centres, self-service or buffet-style dining is commonly encountered. This type of service allows athletes to select various menu options provided in large serving containers (Penggalih et al., 2021). To enhance consumer satisfaction, the accuracy of food packaging should be considered, considering aspects such as the cleanliness of serving utensils, food temperature control, and alignment with athletes’ meal needs (Penggalih et al., 2021) (Rodgers, 2007).

CONCLUSION

The satisfaction rating of athletes at PPLP and SKO regarding the food provision in the athlete dormitories is considered good in several aspects, including the timely serving of meals and the cleanliness and neatness in food packaging, the dining area, and the food handlers. All assessed aspects related to menu variety in PPLP and SKO, both overall and for each type of dish, the variety of food ingredients, portion suitability for athlete needs, meal serving timeliness, packaging

presentation, dining area condition, food handler cleanliness, food appearance, and food taste, are associated with athlete satisfaction. The aspects that have the strongest correlation with satisfaction levels are the timeliness of meal service, food packaging, the cleanliness and tidiness of the dining area, the cleanliness of food handlers, food presentation, and food taste.

SUGGESTION

The correlation between food ingredient variety, menu variety, and athlete satisfaction in meal provision provides a basis for considering ongoing efforts to modify menus and arrange menu cycles to prevent athletes from becoming bored with the food provided. Menu cycles could be extended to longer, such as a 10-day cycle, to offer greater diversity in food options.

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FOOD WASTE AND FOOD SERVICE SATISFACTION AMONG OLDER ADULTS IN NURSING HOMES

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ABSTRACT

Food waste and food service satisfaction can be used of a good food service management. Nursing homes are an institution that also provides food service. This study aims to identify and analyze the association between food waste and food service satisfaction among older adults in the nursing home in Surabaya, Indonesia. A cross-sectional study was conducted on 63 older adults. The 3x24-hour visual Comstock method was used to identify food waste and food service satisfaction was assessed using a questionnaire adapted from the RFSQ (Resident Foodservice Satisfaction Questionnaire) and FoodEx-LTC (Food Expectations-Long-Term Care). Data were analyzed descriptively and inferentially using the Chi-square test. The association between food waste and older adults' food service satisfaction is insignificant ($p=0.115$). The majority of the older adults (71.4%) were satisfied with the food service and the average of food waste was relatively low ($\leq 20\%$). However, some menus still had $>20\%$ leftovers. It is important to investigate older adults' acceptability and preferences of each menu to minimalize food waste. Menu cycle planning needs to be evaluated periodically and well-designed to meet the nutritional needs of the residents.

Keywords: diet, food service satisfaction, food waste, nursing home, older adults

INTRODUCTION

Food service is a series of processes starting from menu planning, purchasing, preparation, processing, and distribution, to serving food till it is ready to be consumed by consumers (Andrini, 2012; Taqhi, 2014). Food service applied in nursing homes is non beneficially oriented that intended to meet the nutritional needs of the older adults in nursing home (Widyastuti et al., 2018). Therefore, the implementation of food service in nursing homes are prone to causing dissatisfaction.

Nursing homes are one of the government programs that are made to support the welfare of the homeless, poor, neglected, or living-alone older adults (BPS, 2021; Rahayu & Ma'ruf, 2018). Several previous studies have stated that older adults who live in nursing homes tend to be malnourished, have a lower BMI, and more likely to experience malnutrition than the older adults who live in the community (Saghafi-Asl & Vaghef-Mehrabany, 2017). Malnutrition is a condition that is often found in older adults (Amarya et al., 2015, 2018). Malnutrition have a higher risk of reduced

mobility, increased risk of falls and fractures, susceptibility to infectious diseases, and in the end, it can worsen the condition of malnutrition (Divert et al., 2015). Therefore, improving food service satisfaction in nursing homes are essential.

Nutritional problems in the older adults are closely related to inadequate intake of nutrients (Divert et al., 2015). Food service satisfaction and food waste are the indicators that can be used to assess food service successes as well as assess intake by assessing the estimated amount of intake (Puspa et al., 2019; Semedi et al., 2013). Adequate nutritional intake in the older adults is important due to its related to minimizing worsening of health and nutritional problems (Sofia & Gusti, 2017).

Food service satisfaction is known to be associated with improved nutritional status related to a more adequate amount of intake and minimal food waste (Semedi et al., 2013). Previous studies showed that the older adults care was institution that had the highest amount of leftover food (20.6%) after canteens, restaurants, and pre-school food service. And also, nursing home had the

highest number of leftovers per portions after restaurants and hotels, which amounted to 129 grams of leftovers per meal (Malefors et al., 2019). This shows that food waste in institutions such as nursing homes need to be considered and related to the food services provided. Therefore, this study aims to analyze the relationship between the level of satisfaction with food service and food waste for the older adults at nursing home in Surabaya.

METHODS

This observational study with a cross-sectional design was followed by the older adults living at nursing home in Surabaya. Population of older adults living at nursing home in Surabaya are 171 including independent, partial, and bed-rest older adults. The sample size obtained is 63 older adults calculated using Lemeshow formula. The sampling method was carried out using the simple random sampling method and justify based on central limit theorem. The sample inclusion criteria was the older adults at least 60 years old, had lived at nursing home in Surabaya for at least 3 months, independent older adults, able to communicate well, cooperative, and did not have memory disorders. The exclusion criteria for this study were the older adults with dementia and the older adults on bed rest.

This research was conducted from 2021 to 2022. The data collected consist of the characteristics of the older adults, food waste, and food service satisfaction. Characteristics data were obtained from older adults and nursing home's database including sex, age, length of stay, education level, medical history, and nutritional status based on BMI. Education level was classified as very low (did not go to school), low (primary or junior high school), moderate (senior high school), and high (college). While BMI was classified refers to Indonesian Ministry of Health classification which are underweight (<18.5 kg/m²), normal (18.5-25.0 kg/m²), and overweight/obese (>25.0 kg/m²) (Kemenkes RI, 2014).

Food waste assessment was carried out with the visual comstock method through observations made by nutritionists and food service satisfaction was carried out using interviews based on questionnaires. Food waste observations were

carried out for three days including breakfast, lunch, afternoon snack, dinner, and evening snack using a 0-5 scale (from 0% to 100% food waste) (BPPSDMK, 2018). Refers to Decree of Ministry of Health number 129/Menkes/SK/II/2008, food waste $\leq 20\%$ can be used as success food services indicator (Dewi, 2015).

The food service satisfaction questionnaire was designed by the references of the RFSQ (Resident Foodservice Satisfaction Questionnaire) by Wright et al. (Wright et al., 2008) and FoodEx-LTC (Food Expectations-Long-Term Care) by Crogan et al. (Crogan et al., 2004). The RFSQ and Food-Ex questionnaires were chosen because they were designed to assess food service satisfaction in nursing home settings. In addition, that questionnaires has been tested in several previous studies.

The data obtained were then analyzed descriptively and inferential analysis using the chi square test. This research has been approved by the Ethics Committee of the Faculty of Public Health, Airlangga University with the number 78/EA/KEPK/2022.

RESULTS AND DISCUSSIONS

A total of 63 older adults people participated in this study. Respondents were dominated by women (69.8%) with an age range of 60-74 years (61.9%). The length of stay of the older adults at nursing home in Surabaya is mostly 12-36 months. The nutritional status of the older adults based on Body Mass Index (BMI) showed that 15.9% were classified as underweight, although the normal nutritional status was recorded at around 50.8%. The characteristics of the older adults can be found in more detail at table 1.

In a food service system, leftovers can be used to assess the estimated amount of food intake. Food waste is influenced by several factors, including external and internal factors. External factors include taste, food quality, limited menu choices, inappropriate portion size and meal times, dining environment, economic, educational, and socio-cultural factors. While the internal factors that influence include appetite, eating habits, age, gender, and illness (Puspa et al., 2019; Simzari et al., 2017).

Table 1. Participant's Characteristics

Variable	n (%)
Sex	
Male	19 (30.2)
Female	44 (69.8)
Age (year)	
60-74	39 (61.9)
74-90	24 (38.1)
>90	0 (0.0)
Length of stay (month)	
<12	19 (30.2)
12-36	28 (44.4)
≥36-60	15 (23.8)
≥60	1 (1.6)
Education level	
Very low	17 (27.0)
Low	38 (60.3)
Moderate	6 (9.5)
High	2 (3.2)
Medical history	
Hypertension	46 (73.0)
Gout/arthritis	17 (27.0)
Diabetes mellitus	12 (19.0)
Stroke	4 (6.3)
Heart disease	1 (1.6)
Other	15 (23.8)
Nutritional status	
Underweight	10 (15.9)
Normal	32 (50.8)
Overweight/obese	21 (33.3)

In this study, the leftovers for three days are relatively small, but in certain menus, the leftovers are still quite high. A lot of leftover food can indicate that less food is consumed. Some respondents said the reason for leaving food was because the menu did not match their eating preferences and the processing method was less favorable. Also, some of them was having their eating preference based on their awareness of medical problem. Sometimes, older adults with underweight status was more likely to ask an additional portion, while overweight/obese older adults had their leftover food. Dietary patterns across generations have been shown to differ in previous studies (Sari et al., 2022). Puspa et al., (2019) states that the quantity of leftovers is much influenced by taste, appetite and the way of presentation (tidiness and cleanliness of cutlery).

Based on the observations, the most food leftovers were found on the third day. Although the average leftover food on the third day was just a few ($\leq 20\%$), it still needs to be noticed that almost half (42.9%) of the respondents have a lot of leftovers. Based on the type of food provided, the largest leftover food was at lunch with a menu of fried catfish and tamarind vegetable soup. Meanwhile, on other days, the most leftovers were found on the first day menu, namely fish sardines (pindang sarden) with 26.0% leftovers. The distribution of leftover food for 3 days menu can be seen at table 2 and table 3.

Beside food waste, consumer satisfaction with food service can also be an indicator of the success of a food service. The quality of food service can be assessed based on several indicators such as food quality (taste and appearance), punctuality, staff service, cleanliness of cutlery, menu variations, and the atmosphere of the dining environment (Nurqisthy et al., 2016; Puspa et al., 2019; Velawati et al., 2021). A better level of satisfaction is associated with a smaller decrease of nutritional status due to higher intake and less food waste (Semedi et al., 2013).

Food service satisfaction of the older adults at nursing home in Surabaya which is shown at table 4, most of the older adults are satisfied with the overall food service provided by nursing home in Surabaya (71.4%). At each indicator, food quality is the indicator that has the most dissatisfaction value (44.4%) compared to other indicators such as punctuality, staff service, cleanliness of cutlery, and the atmosphere of the dining environment.

Food quality, including the taste and appearance of food, contributes to increasing the attractiveness and appetite of consumers. Punctuality of food serving is related to the accuracy of the condition of hunger and satiety that affects one's appetite. Cleanliness of cutlery in fact can also affect someone's appetite. Dirty cutlery can make consumers feel disgusted and have no appetite. In addition, the dining environment and the staff service can affect the mood or psychology of consumers (Nawai et al., 2021; Semedi et al., 2013).

Residents' food satisfaction in this study shows a positive perspective. Most of older adults are satisfied and the most positive indicator is

Table 2. Food Waste on Each Type of Food at Griya Werdha Surabaya

Menu Cycle	Food Waste Mean (%)			Total
	Breakfast	Lunch	Dinner	
Day 1				
Staple food				
Rice	6.4	4.2	4.6	
Noodle soup	-	-	1.0	
Plant-based protein				
Tofu sardines	1.7	-	-	
Fried tofu	-	0.6	-	
Tofu (<i>perkedel</i>)	-	-	2.9	
Animal protein				
Fish sardines	26.0	-	-	
Vegetable				
Mix vegetables (<i>lodeh</i>)	-	11.4	-	
Snack/Fruit				
Banana/melon	-	2.0	-	
Steamed sweet potato	-	-	7.1	
Total				8.4±7.3
Day 2				
Staple food				
Rice	8,1	8,4	6,5	
Plant-based protein				
Tofu	9,3	-	-	
Tofu (<i>opor</i>)	-	-	7,5	
Animal protein				
Meatball	7,1	-	-	
Red soup sausage and chicken	-	18,9	-	
Shred chicken (<i>opor</i>)	-	-	7,1	
Vegetable				
Red soup	-	7,5	-	
Snack/Fruit				
Banana/orange	-	5,6	-	
Pudding	-	-	14,7	
Total				10,4±9,8
Day 3				
Staple food				
Rice	9,6	22,4	7,7	
Plant-based protein				
Fried tempeh	2,0	-	-	
Stew tofu	-	-	5,6	
Animal protein				
Fried catfish	-	45,8	-	
Stew meat	-	-	9,6	
Vegetable				
Stir carrot cauliflower	16,5	-	-	
Tamarind vegetable soup	-	32,0	-	
Snack/Fruit				
Banana/papaya	-	13,9	-	
Steamed sweet potato	-	-	17,9	
Total				17,4±15,9

Note: the use of '-' means it was not on the menu at that meal time.

Table 3. Food Waste at Griya Werdha Surabaya

Day	Mean±SD (%)		Mean±SD (%)
	Few (≤20%)	Lots (>20%)	
1	56 (88.9)	7 (11,1)	8,4±7,3
2	54 (85.7)	9 (14.3)	10,4±9,8
3	36 (57.1)	27 (42.9)	17,4±15,9
Total			12.0±9.0

Table 4. Food Service Satisfaction by the Older Adults

Variable	Satisfied n (%)	Not satisfied n (%)
Food Service Satisfaction Indicators		
Food quality	35 (55.6)	28 (44.4)
Punctuality	55 (87.3)	8 (12.7)
Staff service	54 (85.7)	9 (14.3)
Cutlery cleanliness	49 (77.8)	14 (22.2)
Dining environment	51 (81)	12 (19)
Overall food service satisfaction	45 (71.4)	18 (26.8)

Table 5. Relationship between Food Waste and Food Service Satisfaction

Food Waste	Food Service Satisfaction		p value
	Not Satisfied n (%)	Satisfied n (%)	
Few	12 (19.0)	38 (60.3)	0.115
Lots	6 (9.5)	7 (11.1)	

food quality. This is in line with previous study that indicated indicators of food quality are the most influential aspects of patient satisfaction in general, while the indicator that is considered the most positive is the service of the staff (Messina et al., 2013). This is different from this study which shows that the indicator with the most positive value is the punctuality indicator.

The high satisfaction rating of the older adults at nursing home in Surabaya can be attributed to the different backgrounds of the older adults. Based on the results of interviews, most of the older adults are neglected or living-alone so that can affect the older adults by being more accepting of the situation and grateful for having a better life than outside the nursing home. In addition, most of the older adults have lived in the nursing home for 1-3 years so that they have more or less adapted well to the conditions in the nursing home.

Based on table 5, this study shows that there is no relationship between food waste and food service satisfaction ($p=0.115$). Basically, satisfaction can be presented in food waste (Simzari et al., 2017). Previous studies have stated that food service satisfaction is related to the level of consumer consumption (Divert et al., 2015; Heidi et al., 2017; Navarro et al., 2016). The higher the level of satisfaction, the less nutrients are lost, which means there is less leftover food. This study is not in line with the results found by Ronitawati et al. (Ronitawati et al., 2021) which shows that there was a relationship between the level of satisfaction with the value of missing nutrients ($p=0.0001$, $r=-0.34$).

The absence of this relationship might be caused by the good results of the older adults' food satisfaction that mostly positive. Length of stay was one of the institutional service satisfaction predictors. Residents with a longer length of stay had better life adjustments (Sun et al., 2020). Whilst for the food waste, in this study was more influenced by the residents' food preference and mostly high only in some specific menus.

Food service in nursing home is one factor that can affect the nutritional status of the older adults related to capability to provide nutritional needs of the older adults every day. Dissatisfaction with food service can increase the risk of malnutrition up to 20 times. The more satisfied the older adults with food service are, the better the nutritional status (Saghafi-Asl & Vaghef-Mehrabany, 2017). Nursing home in Surabaya allows the older adults to receive food from outside nursing home but they do not allow the older adults to buy food from outside. Frequency of relatives or family's visit can be considered quite rare, so most of the nutritional needs is provided by the nursing home. Therefore, the nursing homes must provide a proper menu that can fulfill the older adults' requirement.

The limitations of this study was the assessment of food service satisfaction is very subjective and influenced by the level of adaptation of the older adults to life in nursing home even though it has been carried out by direct interviews without the presence of the nursing home's staff. High food waste in this study tends to be more directed to the taste, preferences, and mood of the older adults. Besides that, short sample size in this

study was due to the inclusion criteria that only enroll independent' older adults which were only around 100 and must be excluded again with the other criteria. Further studies need to be done with a bigger scope area, not only in one nursing home, to avoid a biased result.

CONCLUSION

Food service satisfaction and food waste are important things to consider in the food service management system, especially for the older adults. There is no relationship between leftover food and food service satisfaction for the older adults at nursing home in Surabaya, this possibly due to the subjective answer of the older adults satisfaction in terms of their gratitude of being care in nursing home rather than living alone. Although the satisfaction score is good, there is a high amount of leftovers food on some of the menus served, so it is necessary to explore the acceptability of each menu served and older adults' preferences to minimize the amount of food waste. In addition, the menu cycle planning needs to be evaluated periodically and arrange by adjusting to the nutritional needs of the older adults.

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GUIDELINE FOR AUTHOR

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Papers submitted to the editorial are self-generated papers, scientific, contain contemporary issues and unpublished. To avoid duplication, the editor does not accept papers that are also sent to other journals at the same time for publication.

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III. WRITING SYSTEM

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Results and Discussion

Conclusion and Suggestion

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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

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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..

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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).

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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

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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

Smith (2017) or (Smith, 2017)

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

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

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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:

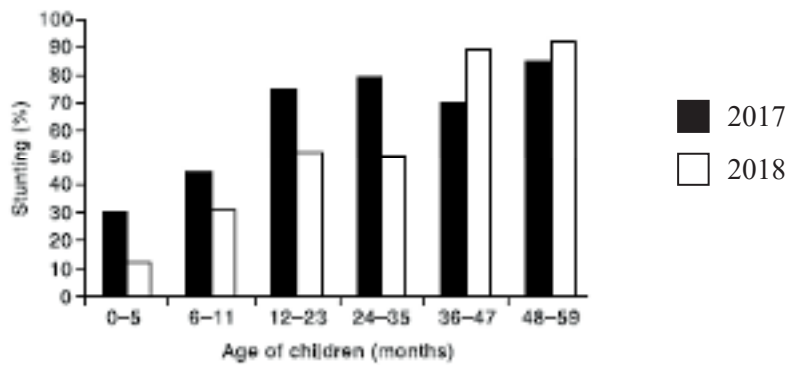


Figure 1. Changes in Stunting Prevalence (%) in Toddlers in Kalimantan

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FISH-BASED COMPLEMENTARY FEEDING PRACTICES INCREASING MACRO AND MICRO NUTRITION INTAKE AND HEMOGLOBIN LEVELS IN ANEMIA TODDLERS

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ABSTRACT

Iron deficiency anemia in toddlers can be prevented by introducing complementary foods alongside breastfeeding. Fish, rich in protein and iron, offers a valuable nutritional source for young children, but proper processing is crucial. This research investigated the impact of fish-based complementary food processing practices (known as MP-ASI in Indonesia) on the nutritional intake and hemoglobin levels of children under five in Tiro District, Pidie Regency. Using a quasi-experimental design, this study involved 40 toddlers from the stunting-prone area of Tiro District, Pidie Regency, along with their mothers as respondents. The toddlers, aged 12-36 months without severe illnesses, were included in the study. The intervention comprised educating and practicing complementary food processing. Before-and-after intervention comparisons showed significant improvements in energy, carbohydrate, protein, zinc, and iron intake levels. Additionally, there was a notable increase in the average hemoglobin (Hb) level, rising by 1.06 g/dl after one month of fish-based complementary food processing intervention. In summary, this intervention effectively enhanced the nutritional quality of children's intake and increased their hemoglobin levels, contributing to the prevention of iron deficiency anemia in toddlers.

Keywords: anemia, nutritional intake, hemoglobin, fish consumption, education

INTRODUCTION

One of the priorities of the Program Indonesia Sehat through a Family Approach is reducing the prevalence of stunting. Stunting (low height for age) affects about a quarter of children aged <5 years worldwide (Gharpure et al., 2021). The problem of stunting in toddlers is the main nutritional problem faced in Indonesia.

According to SSGI 2022, Indonesia has a stunting prevalence of 21.6%. This figure is still above the threshold set by WHO, which is 20%. Based on national data, Aceh Province is ranked 5th in the highest prevalence of toddlers after East Nusa Tenggara Province and West Sulawesi Province. The success that the Aceh Government has achieved in reducing the stunting rate from 41.5% in 2013 to 37.3% in 2018 to 31.2% in 2022 (Kemenkes RI, 2014, 2018, 2022).

Stunting is a condition where children under five have a low height for their age. Stunted children experience malnutrition, which affects the maturity of nerve cells. Stunted children experience abnormal developmental delays such as slow motor movements, reduced intelligence, and slow social responses (Rosyidah et al., 2021). Several factors influence stunting, namely the age of the child, family size, number of children under five years in the household, wealth status, source of drinking water, eating habits, and food insecurity (Mengesha et al., 2021).

Previous studies stated that eating habits are one of the factors related to the incidence of stunting (Mulyaningsih et al., 2021). Eating habits affect the nutritional intake of stunted children. Hendraswari et al., pada tahun 2021 found that the intake of macronutrients such as energy and

protein intake is the main factor influencing the incidence of stunting. In addition, low iron is also a factor associated with stunting. Stunted toddlers generally have lower hemoglobin levels than toddlers who are not stunted (Flora et al., 2019; Losong & Adriani, 2017).

Nutrient intake for stunted toddlers can be done by providing additional food as a complementary food for breast milk. Providing additional food can increase nutritional intake, especially increasing hemoglobin levels (F. Wang et al., 2017; J. Wang et al., 2017). One additional food source of iron is derived from animal food sources (H.J. et al., 2011). Fish is a source of animal food with good nutritional content, especially a source of protein and iron (Safitri & Puspita, 2018; Syahril et al., 2016). Fish can also be used as a variety of innovative products such as biscuits, food bars, and cookies to address the problem of malnutrition, especially stunting, which can improve malnutrition status in children under five (Darawati et al., 2021; Susyani et al., 2022; Yuliana et al., 2019).

Based on this, the authors conducted this study to analyze the effect of fish-based complementary breastfeeding (in Indonesia: MP-ASI) processing interventions on the intake of macronutrients (Carbohydrate, protein, fat) and micronutrients (Vitamin A, C, calcium, zinc, and Fe) also Hemoglobin of toddlers. This activity was carried out in the stunting locus area designated as a sub-district with a high category of nutritional problems in Aceh, namely Tiro District, Pidie Regency.

METHOD

This type of research used a Quasi-Experimental one-group pretest-posttest design, where the research design was to compare before and after the intervention was given. This research was conducted in the Stunting Locus area, Tiro District, Pidie Regency, in July-August 2022.

The initial stage of this research was sampling, such as selecting villages, posyandu (an integrated health center), and households. The research subjects consisted of toddlers aged 12-36 months in the stunting locus area, Tiro District, Pidie Regency. There were 5 locus of stunting villages in the working area of the Tiro Health Center, namely

Pulo Masjid Village, Peuneudok Village, Pulo Siblah Village, Panton Beunot Village, and Panah Village. Sample calculations in this study using the Lemeshow formula obtained a minimum number of 36 people. Furthermore, respondents were taken by consecutive sampling by taking toddlers who met the inclusion criteria and obtained a total of 40 samples. Subject/sample inclusion criteria were toddlers aged 12-36 months, not currently seriously ill, their mothers, or respondents willing to participate in research activities up to signing informed consent.

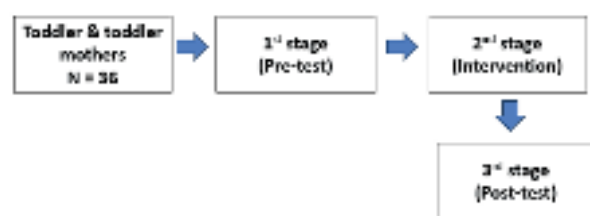


Figure 1. Research Stages

This research consisted of several stages: pretest/baseline, intervention I and II, and posttest (Figure 1).

1. Stage I, namely baseline data or pretest. At this stage, baseline data were collected, including data on household socioeconomic characteristics, subject characteristic data, macronutrient intake data (carbohydrates, protein, fat), and micro (Vitamins A, C, calcium, zinc, and iron) from subjects taken through 24-hour food recall method for toddlers. Meanwhile, data on blood hemoglobin levels were known in the fingertip capillaries using the digital easy touch method.
2. Stage II, the intervention was carried out 2 times during 1 month, 2 times 45 minutes each. The interventions carried out were in the form of nutritional counseling and behavior change practices in the form of processing fish-based complementary breastfeeding with demonstrations of processing fish-based complementary breastfeeding under the rules or principles of processing complementary breastfeeding for toddlers (guide in the recipe book). At the same time, the practice of processing fish-based complementary breastfeeding which was practice, was The practice of processing fish was carried out following the recipes contained in the recipe pocketbook. Nutrition education intervention

steps for respondents and posyandu cadres are as follows:

- a. Team opening
 - b. Administration of questionnaires before intervention
 - c. Collecting completed questionnaires
 - d. Explanation of material by the research team or resource persons, including 1) IYCF (Toddler Feeding); 2) problems of stunting and anemia; 3) the importance of fish in the growth and development of toddlers and fish-based complementary breastfeeding processing. Explanation of material using lecture and discussion methods using infocus tools, leaflets, and posters.
 - e. The team's practice of processing fish-based complementary breastfeeding included processing fish-based complementary breastfeeding menus high in nutrients to support growth and increase blood hemoglobin levels to prevent stunting and anemia. All participants would be given a fish-based complementary breastfeeding processing recipe book and leaf leads related to anemia in children under five.
3. The next stage was the posttest. The posttest stage consisted of collecting data on macronutrient intake (carbohydrates, protein, fat) and micronutrient intake (Vitamins A, C, calcium, zinc, and iron), blood hemoglobin levels in fingertip capillaries using the digital easy touch method.

The nutrient intake data collected was first converted to weight in grams, then the nutritional content was calculated, such as energy (kcal), protein (grams), fat (grams), calcium (grams), iron (mg), vitamin C (mg), and vitamin A (μg) using the Nutri-Survei 2007 program software. Then, it was compared with the nutritional adequacy figures for each toddler.

Microsoft Excel 2007 and SPSS 16.0 for Windows were used to analyze data descriptively and inferentially. Before testing the data in SPSS, the data normality test was first performed using the Kolmogorov-Smirnov test. The test for differences in normally distributed numerical data uses Paired T Test analysis to analyze the differences before and after the intervention of fish-based complementary breastfeeding processing practices on the adequacy of macro and micro-nutrients and hemoglobin levels of children under

five. Commission on Research Ethics Involving Human Subjects Number: LB.01.03/6/5621/2022.

RESULTS AND DISCUSSION

The sample characteristics in this study consisted of age, gender of the toddler, birth weight and height, and health history (Table 1). The sample in this study were toddlers aged 12 months to 36 months who were selected to meet the criteria, namely as many as 40 people. Most of the toddlers are in the age range of 12-24 months, namely 80.0%, male (60.0%), do not have LBW (Low Birth Weight) status (92.5%) and with birth length >45 cm (57.5%).

Children with birth weight lower than 2,500 g had a 5.9 times the risk of becoming stunted compared to children born weighing more than 2,500 g ($p < 0.05$; 95% CI: 0.93–37.8). Furthermore, children with birth length below 48 cm (short) had a 15.0 times higher risk of experiencing stunting ($p < 0.05$; 95% CI: 2.58–87.9) compared to children born with body length over 48 cm (Lukman et al., 2021). The child's birth weight and birth length are greatly influenced by the nutritional status and health of the mother before and during pregnancy, which affects the growth and development of the fetus during the neonatal period. Birth weight and length are closely related to the child's growth after birth. It was further explained that newborns with low birth weight have a higher risk of death in the first 28 days of life. Surviving people are more likely to suffer from stunted growth and lower IQs (Barker, 2007).

Table 1. Distribution of Toddler Characteristics

Toddler Characteristics	Number of toddlers	%
Age		
12-24 months	32	80.0
25 -36 months	8	20.0
Gender		
Male	24	60.0
Female	16	40.0
LBW status		
LBW (≤ 2500 g)	3	7.5
Not LBW (> 2500 g)	37	92.5
Birth length		
≤ 45 cm	17	42.5
> 45 cm	23	57.5

Intake of essential nutrients is related to the need for adequate health. The intake needs for several nutrients that must be met come from the food consumed. Nutritional adequacy is a comparison between nutritional needs and the intake of a particular individual or population, expressed as a percentage of recommended nutritional adequacy (Damara & Muniroh, 2021; Yuniar & Mahmudiono, 2022).

The sample nutrient intake data in this study were compared with the Nutrition Adequacy Rate based on the age of each toddler. The results obtained can be seen in Table 2.

Table 2. Average Adequacy Level of Energy and Other Nutrients Samples Before and After Intervention

Adequacy level	Pretest		Posttest		p-value
	Mean	SD	Mean	SD	
Energy (%)	43.3	18.75	60.9	14.2	0.00*
Carbs (%)	31.4	14.4	46.5	17.5	0.00*
Protein (%)	136.6	54.30	162.3	43.9	0.02*
Fat (%)	55.3	32.8	56.0	32.6	0.89
Vit A (%)	149.6	247.6	113.4	182.8	0.36
Vit C (%)	16.9	24.8	25.3	24.8	0.15
Calcium (%)	38.0	34.9	48.20	48.2	0.06
Zink (%)	94.6	54.4	122.0	50.9	0.00*
Fe (%)	52.1	27.3	66.8	32.5	0.00*

Table 2 shows the level of nutritional adequacy of the toddler samples in the study, most of whom were deficient or lacking, below 70% compared to the Nutrition Adequacy Rate. Only the average protein, vitamin A, and zinc values showed good results. The Paired T-test tests showed significant differences in the mean values before and after the intervention on the level of adequacy of energy, carbohydrates, protein, zinc, and iron. While the rest are not significantly different.

Fish is a source of animal food which is rich in nutrients. As a highly nutritious food, fish consumption is highly recommended for children for normal growth and development (Maulu et al., 2021). Fish has a high protein content with complete amino acids, so it can be developed as a good additional food for toddlers (Kadir, 2021). The high nutritional content in fish is

protein, calcium, oleic, palmitic, linoleic, and stearic acids. The highest amino acids are aspartic acid, glutamic acid, arginine lysine, and leucine (Nuryanto et al., 2022). Besides that, fish is also rich in micronutrients such as vitamins and minerals. Complementary foods substituted with fish have higher vitamin A and zinc content than complementary foods not mixed with fish (Hope et al., 2021).

Foods of animal origin derived from water, such as fish, can provide important nutrients and bioactive factors for human health. Animal food sources in the form of fish contain many nutrients such as docosahexaenoic acid [DHA], choline, vitamin B12, iron, and zinc. (Iannotti et al., 2022). Previous studies have shown that there is an increased effect of giving fish to anemic toddlers with a frequency of consumption of 7 times a week can improve the anemia status of children with indicators of increased blood profile in the form of ferritin, serum transferrin, and Hb. The existence of nutrition education interventions and fish-based complementary breastfeeding processing practices in this study can encourage mothers of toddlers to increase giving fish to their children. Intervention in giving fish to children who are anemic affects higher Hb repair and lower serum transferrin (Werner et al., 2022).

Hemoglobin levels

Test toddlers' Hb levels were measured twice before and after providing educational interventions and fish-based complementary breastfeeding processing practices. In Table 3, it can be seen that most of the samples of toddlers had anemia status, both before (87.5%) and after the intervention (77.5%). However, several samples showed a slight decrease in anemia status after providing nutritional education interventions and fish-based complementary breastfeeding processing practices.

Table 3. Anemia Status and Average Hb Levels of Samples Before and After Intervention

Anemia Status	Pretest		Posttest	
	n	%	n	%
Anemia	35	87.5	31	77.5
Not Anemia	5	12.5	9	22.5

Micronutrient deficiencies and stunting are significant problems in most children aged 6-59 months. The average Hb level of the samples before and after the intervention was in the low or anemia category (Ernawati et al., 2021). The incidence of anemia in stunted children is largely due to low consumption of animal foods (Mohammed et al., 2019). The average Hb level before the intervention was 8.64 g/dL and increased to 9.70 g/dL after the intervention. There was an increase in the average Hb level after giving counseling interventions and fish-based MP-ASI processing practices for 1 month of 1.06 g/dL. Even though there was an increase in posttest Hb levels, the average Hb level was in the anemia category.

Table 5. Average Hb Levels of Samples Before and After Intervention

Hb Level	Mean	SD	Mean	SD	p-value
Hb level	8.64	2.40	9.70	1.51	0.00*

The results of the different tests in this study used the Paired T-test. Table 4 shows a significant difference in the mean values of pretest and posttest Hb levels ($p=0.000$; $p>0.05$) before and after receiving nutritional education interventions and fish-based complementary breastfeeding processing practices. Anemia and stunting are closely related to a synergistic improvement in child health (Gaston et al., 2022). Previous studies have shown that most stunted children have a significant relationship with high anemia status (Rahman et al., 2019). Anemia is a comorbid factor in stunting that negatively impacts later life (Orsango et al., 2021). High anemia status in stunting toddlers is associated with increased cognitive abilities and lower physical growth of children (Orsango et al., 2021; Tampy, 2020). Demographic, socioeconomic, and geographic characteristics are also important drivers of stunting and anemia in children under 5 years (Gaston et al., 2022).

Factors of nutritional status and health can cause anemia in children. Malnutrition may not be directly related to anemia, but it leads to certain changes in the body that make it susceptible to health hazards that can cause anemia. Children and women who suffer from malnutrition are more likely to have a weaker immune system, making

them susceptible to various diseases and health hazards such as parasitic infections or chronic inflammation. Conditions like this can reduce hemoglobin levels in the blood, which causes an increase in the prevalence of anemia (Lukman et al., 2021). Stunted children of anemic mothers are at high risk for anemia (Lukman et al., 2021). This is explained based on certain factors that influence anemia and stunting. For example, mothers and children can have a healthy diet and access to micronutrient food sources rich in iron. Apart from that, they share the same things in the environment, have access to the same health facilities, and tend to have the same genetic characteristics. To overcome the problem of anemia in children, it is vital to design programs that target mothers and children. Empowering women or mothers of toddlers is one way to reduce the high prevalence of anemia and malnutrition in toddlers (Lukman et al., 2021).

CONCLUSION

There were significant differences in the average energy, carbohydrate, protein, zinc and iron intake values before and after the intervention. Increased energy intake, carbohydrates, protein, zinc, and iron were obtained after administering the intervention. There was an increase in the average Hb level after giving counseling interventions and fish-based complementary breastfeeding processing practices for 1 month of 1.06 g/dL. Further studies are needed with larger subjects and an extended number of intervention days to see the impact on anemia status.

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NUTRITION EDUCATION AND ASSISTANCE BASED ON BEHAVIOR CHANGE IN ADOLESCENT GIRLS TO IMPROVE NUTRITIONAL STATUS AND HEMOGLOBIN LEVELS

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ABSTRACT

Inappropriate nutritional behavior is a common cause of nutritional issues among adolescents, often leading to conditions like underweight and anemia. This study aimed to assess the impact of nutrition education and behavioral change-based support on the nutritional status and hemoglobin levels of adolescent girls. The study included 75 adolescent girls aged 16-18 years from Singosari Village, Gresik District, chosen through purposive sampling. Over a 30-day period, intensive nutrition education and assistance were provided, utilizing various methods such as lectures, discussions, role play, and practical exercises. The results revealed significant improvements in various aspects. Participants exhibited increased nutrition knowledge, energy and protein intake, body weight, nutritional status, and hemoglobin levels after the intervention ($p < 0.05$). The rise in knowledge was linked to higher protein intake ($p=0.009$), while adequate protein intake was associated with weight gain ($p=0.025$). Weight gain, in turn, correlated significantly with improved nutritional status based on BMI/U ($p = 0.041$). Hemoglobin levels also significantly increased, shifting from 11.6 ± 0.2 mg/dl (low) to 13.4 ± 0.1 mg/dl (normal) after the intervention. This improvement in hemoglobin levels was linked to increased energy intake ($p = 0.012$) and a change in the practice of consuming iron supplementation, shifting from no usage to weekly consumption. In conclusion, nutrition education and behavioral change-based support can effectively enhance energy, protein, and iron intake, leading to improved nutritional status and hemoglobin levels among adolescent girls.

Keywords: adolescent, education, assistance, hemoglobin, nutrition

INTRODUCTION

The nutritional status of women before conception is one of the influential factors during pregnancy and the health of the baby to be born. Malnutrition in pregnant women can be the cause of babies born having low birth weight and malnutrition in toddlers (Rahma and Nuradhiani, 2019). Malnutrition in pregnant women can also increase the risk of maternal death (UNICEF, 2018). The nutritional status and health of pregnant women are determined since adolescence or during their childbearing age (WUS) (Waskara, 2021). Currently, adolescents in Indonesia face three nutritional problems at once, including macronutrient deficiency (short and thin), micronutrient deficiency (anemia), and

overweight (overweight and obesity) (Baroroh et al. 2022).

Basic Health Research Data (RISKESDAS) in 2018 showed that the prevalence of short and very short in adolescents aged 13-15 years touched 25.7% while adolescents aged 16-18 years were 26.9%. Similarly, the prevalence of underweight and very thin adolescents, recorded as many as 8.7% of adolescents aged 13-15 years and 8.1% aged 16-18 years have underweight and very thin nutritional status. In contrast, the prevalence of adolescents with overweight and obese nutritional status aged 13-15 years was 16.0% and 13.5% aged 16-18 years. In addition to macronutrient problems, the problem of micronutrient deficiency such as anemia in adolescents in Indonesia is also fairly

high. The prevalence of anemia in adolescents is 32% (Ministry of Health RI, 2018).

Adolescents are prone to nutritional problems. Biologically, adolescents need more intake for organ growth and maturation (Bahar et al., 2020). Especially adolescent girls who periodically experience menstruation, so they are at greater risk of anemia (Hidayati et al. 2019). UNICEF (2017) collected data and obtained results, there were changes in consumption patterns and physical activity in adolescents, including adolescents doing the wrong food restrictions because they want to maintain weight, liking contemporary foods that tend to be high in calories and fast food, and the tendency of adolescents to choose foods that are not diverse are factors that play a role in three nutritional problems in Indonesian adolescents. There are also other supporting factors are social and educational inequality, to mental health problems.

UNICEF Indonesia in collaboration with the Government of Indonesia has begun pioneering a youth nutrition program designed to address three adolescent nutrition problems. This program applies a life cycle framework that aims to break the inter-generational chain of malnutrition. The program consists of three types of interventions, there are iron supplementation tablet containing iron and folic acid on a weekly basis to prevent anemia, nutrition education and behavior change. Providing nutrition education or nutrition education is one of the right methods to share information for adolescents and is supported by intensive mentoring which is an effort to change adolescent behavior. Research by Walilulu et al (2018), revealed that nutrition education has an effect on increasing knowledge and efforts to prevent nutritional problems. This study aims to examine the effect of education and nutrition assistance based on behavior change in adolescent girls on improving nutritional status and hemoglobin levels.

METHOD

The research design is quasi-experimental with pre- and post-one group design. This study involved 75 adolescent girls selected by purposive sampling with inclusion criteria aged 16-18 years,

physically and mentally healthy, not on a certain diet / strict diet and came from Singosari village, Gresik. The research began from July to September 2022 in Singosari Village, SMK DharmaWanita and SMK Semen Gresik. Interventions in the form of providing TTD once a week, education and mentoring are carried out for 30 days on active days and holidays with monitoring and evaluation carried out once a week. The main points of nutrition education provided are about the control and prevention of anemia, healthy eating behavior for adolescents. The material was delivered by researchers using the method of lectures and discussions at school gradually once a week for one month (30 days) with details of 4 times the provision of educational materials as well as 4 times taking blood added tablets together in class. Nutrition assistance is focused on monitoring iron supplementation tablet consumption practices and changes in healthy food consumption patterns. Blood tablets are given once a week and taken together in class. The actual consumption of respondents before the intervention was collected using the 24-h-recall method, then monitoring of respondents' consumption patterns was carried out by recording food (food record) and sending photos of food consumed through Whatsapp groups then recorded by enumerators. The results of recording Data collected during the study included the level of knowledge assessed through pre-test and post-test. Pre-test assessment is carried out before education and assistance and post-test is carried out at the end after the last intervention. In addition, consumption patterns, nutritional status (BMI / U) and hemoglobin levels. The tools used include questionnaire questions, 24-h-recall form and estimated food record, weight scale, microtoa and Easy Touch GCHb 3 in 1 hemoglobin test kit to measure Hb levels. Measurement of Hb levels was carried out by the research team. The stages of data analysis collected include normality tests, paired t tests to test for differences between before TTD, education and assistance and after intervention. Next, test pearson correlation to test the relationship between variables. This research has passed the ethical feasibility test by the ethics commission of Universitas Muhammadiyah Gresik No. 124/KET/II.3.UMG/KEP/A/2022.

RESULTS AND DISCUSSION

Characteristics of Respondents

A total of 75 young women were selected as respondents in this study. By age, 46.6% of respondents were 16 years old, 36.7% were 17 years old and 16.7% were 18 years old. All respondents came from Singosari Village, which is one of the stunting loci in Gresik Regency.

Nutritional Knowledge

Adolescence is a transition period from childhood to adulthood which includes biological changes, psychological changes, and social changes (Wulandari, 2014). Some studies say that education or counseling is one effective way to introduce balanced nutrition consumption patterns for adolescent girls (Ningsih, 2018). The knowledge gained by young women through education will influence attitudes, which then determine their behavior (Najahah, 2018; Nugraha et al. 2021).

The level of nutritional knowledge in this study was measured through pre-test and post-test results. As many as 58.7% of respondents had a lack of knowledge before being educated. After intensive education and assistance, nutritional knowledge increased significantly until 100% of respondents had a good level of knowledge ($p = 0.0001$) (Figure 1).

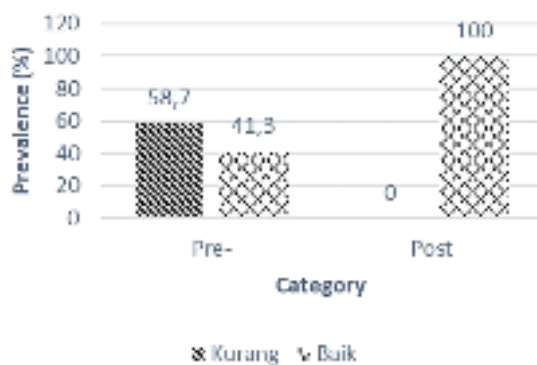


Figure 1. The Level of knowledge of respondents before and after the intervention

In this study, the intervention focused on intensive education and assistance related to behavior change. Education is aimed at increasing knowledge from adolescent girls related to healthy eating behavior and control and prevention of anemia for adolescents so that adolescents can live healthy, grow optimally and be free from anemia. Community-based nutrition education

methods have been widely applied with the aim of improving healthy living behaviors, preventing the emergence of diseases and improving the degree of health). Research by Jamaluddin et al (2020) shows that community-based nutrition education has positive results in the diversity of consumption patterns, hemoglobin levels, and children's attendance at school.

Knowledge is one of the domains that influence human behavior. Changes in behavior based on knowledge will be more consistent than behavior that is not based on knowledge (Notoadmojo, 2013). In practice, respondents with good knowledge do not always have good healthy living practices due to internal and external influences (Veriza & Riyadi, 2018). In this study, intensive assistance was applied as an effort to monitor good behavior that had been conveyed during education. Efforts made include respondents being given a food notebook, every meal students were asked to send photos of food consumed. If there is a discrepancy, respondents are motivated and counseled to meet needs according to their abilities.

Consumption Patterns

The results of recording energy intake before the intervention showed that as many as 66.7% of respondents had energy intake with the category of severe deficit, 9.3% moderate deficit, 8% mild deficit and 16% sufficient with the average energy intake of respondents as 1201 + 34.6 kcal. Not much different from energy intake, more than 50% of respondents protein and carbohydrate intake were categorized as weight deficit (Table 1). This can be the cause of growth and development of young women is not optimal.

Table 1. Classification of respondents' energy and nutrient intake before intervention

Category	Energy Intake (%)	Protein Intake (%)	Fat Intake (%)	Carb Intake (%)
Severely Deficit (<70%)	66,7	56	9,3	70,7
Moderate Deficit (70-79%)	9,3	22,7	4	13,3
Mild Deficit (80-89%)	8	13,3	6,7	8
Adequate (90-120%)	16	8	52	8
Over intake (>120%)	0	0	28	0

Adolescents need a greater intake of nutrients than during childhood. However, teenagers tend to have a diet that is not in accordance with needs. After being educated and mentored for one month, there was a significant increase in energy, protein, fat and carbohydrate intake (Table 2).

Table 2. Results of recording respondents' food intake (24-h recall and estimated food record)

Category	Pre-	Post-	Δ	P value
Energy intake (Kkal)	1201 ± 34,6	1334 ± 37,9	133 ± 15,2	0,0001*
Protein intake (g)	45,6 ± 0,7	50,2 ± 0,9	4,5 ± 0,7	0,0001*
Fat intake (g)	51,0 ± 1,2	55,0 ± 1,2	3,9 ± 0,8	0,0001*
Carb. intake (g)	164,8 ± 9,6	171,4 ± 5,4	6,5 ± 7,6	0,393

Keterangan : uji *t* berpasangan signifikan pada $\alpha < 0,05$

Young women's energy intake increased after education and mentoring by 133+ 152 kcal (Table 2). The increase in total energy cannot be separated from the increase in intake of energy-contributing nutrients, namely protein ($\Delta=4.5 + 0.7$ g), fat ($\Delta=3.9 + 0.8$ g) and carbohydrates ($\Delta=6.5 + 7.6$ g). The increase in protein intake occurred due to changes in the selection of vegetable and animal protein sources that previously rarely consumed vegetable protein to choose fried tempeh as a source of animal protein with a frequency of 4-6 times per week.

The selection of animal protein sources from respondents also changed, which previously on average liked sausages (ready-to-eat foods) consumed 4-6 times per week turned into various processed chicken. Based on data on the composition of Indonesian food, the protein content of sausages is lower than processed chicken. The protein content in 100 g of sausage is only 14.5 g while in fried chicken has a protein content of between 31-35 g per 100 g depending on the body part of the chicken (thigh = 32.1 g; wings = 35 g and breast = 34.2 g). The amount of protein content in processed chicken is twice as much compared to sausages. Consumption of protein that is less and not diverse will greatly impact the condition of growth and development of adolescents (Wulandari, 2014).

Fat intake in respondents also increased. This is related to an increase in protein intake, where the source of protein is also a source of fat. Ready-to-eat sausages sold in the market contain 27 g of fat per 100 g while chicken has various contents according to its part (thigh = 15.7 g; wings = 15.2 g and breast = 16.2 g). Although chicken seems to have less fat content than sausage, the most common chicken processing is fried (such as crispy chicken, chicken katsu, geprek chicken) so as to increase the amount of fat intake from the oil used. Based on the oil absorption table, the absorption of cooking chicken oil is 16%.

Teenagers are one of the groups that dominate the consumption of ready-to-eat foods. The high consumption of ready-to-consume food products is associated with practical products and delicious taste (Godatwar et al. 2015). The level of nutritional knowledge has an impact on a person's attitude and behavior in food selection and the formation of eating habits. Lack of nutritional knowledge about a good diet and lack of understanding of the role of nutrients from various types of food will cause problems not only in nutritional status and health, but also affect intelligence and productivity (Soekirman, 2011).

The nutritional status of adolescent girls is determined by BMI / U which begins with measuring the weight and height of respondents. The average body weight of respondents before the intervention was 53.4 + 2.1 kg. After following the intervention there was a significant weight gain to 54.5 + 2.02 kg ($p = 0.0001^*$) (Table 2). The height measurement result was 1.56 + 0.01 m and did not change between before and after the intervention.

Table 3. The results of anthropometric measurements

Variabel	Pre	Post	P value
BW (Kg)	53,4 ± 2,1	54,5 ± 2,02	0,0001*
Height (m)	1,56 ± 0,01	1,56 ± 0,01	1

Keterangan: uji *t* berpasangan signifikan pada $\alpha < 0,05$

The results of nutritional status classification based on BMI / U before the intervention showed that as many as 42.3% of respondents were underweight, 26.7% Normal, 24% overweight and 8% obese. After the intervention, the prevalence of underweight adolescent girls decreased to 34.7%

and those with normal nutritional status increased to 33.3% (Table 3).

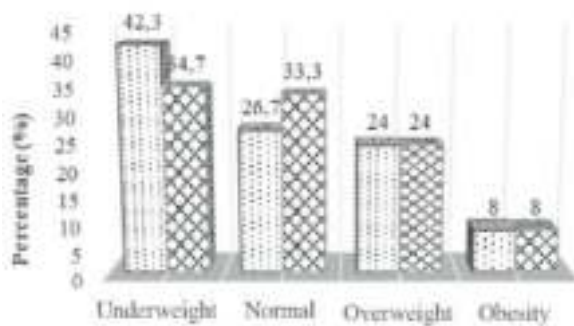


Figure 2. Classification of nutritional status of respondents before and after the intervention

Weight gain is associated with improved nutritional status based on BMI/U ($p = 0.04^*$). The diet of adolescent girls will determine the amount of nutrients needed for growth including weight gain (Mokoginta et al. 2016). Weight gain is also linear with increasing BMI values, so that improvements in nutritional status can be achieved.

The number of overweight and obese adolescent girls did not change between before and after the intervention. This is because research focuses more on improving the quality of consumption (sufficient quantities and diverse types) but does not increase physical activity. The message of the Nusantara Movement to Press Obesity Rates (GENTAS) from the Ministry of Health in an effort to reduce the prevalence of more nutrition is to regulate diet, one of which is the amount of food sources of protein equivalent to food sources of carbohydrates, increase consumption of fruits and vegetables besides that it also increases body movement (physical activity and physical exercise) (Ministry of Health RI, 2017).

In this study, there was an increase in the amount of protein intake as well as an increase in the frequency of fruit and vegetable consumption. Before the intervention, only 30% of respondents consumed fruit daily. After intensive education and assistance, the number of respondents who consumed fruit every day increased to 47% both directly consumed and consumed in the form of juice. As for vegetable consumption, as many as

23.7% of respondents consumed vegetables every day before the intervention and increased to 40%.

Recommendations for fruit and vegetable consumption in someone who experiences more nutrition are at least equivalent to protein and carbohydrates. Consumption of fruits and vegetables in greater quantities has a dual function, namely as a source of fiber and a source of vitamins and minerals. The fiber content in fruits and vegetables can provide satiety in a longer time and can help the body eliminate piles of food in the large intestine. Vitamins and minerals in vegetables and fruits are useful for maintaining health, especially counteracting free radicals due to excess fat accumulation in people who are obese (Ministry of Health RI, 2017).

Hemoglobin Level

The results of measuring respondents' hemoglobin levels before the intervention showed that as many as 62.7% of respondents had anemia with an average hemoglobin level of respondents 11.6 ± 0.2 g / dL. After education and assistance and regular consumption of blood-added tablets once a week, the average hemoglobin level increased significantly to 13.4 ± 0.1 g/dL ($p = 0.0001^*$). The Pearson correlation test showed that increased hemoglobin levels were significantly associated with increased energy intake ($p = 0.012^*$), and were associated with increased TTD consumption. After this research was completed, the task of monitoring TTD consumption was returned to the school.

Research Susanti et al. (2016) explained that iron supplementation on a weekly basis has the same effectiveness in increasing hemoglobin levels in adolescent girls with daily consumption of TTD during menstruation. Giving TTD to adolescents is recommended once a week with an excess level of adherence to higher supplement consumption.

CONCLUSION

Based on the results of the study above, providing education and nutritional assistance based on intensive behavior change can increase knowledge, increase energy, protein, fat and carbohydrates and regular consumption of TTD once a week. The improvement of knowledge

and daily consumption patterns and blood-added tablets is significantly related to improvements in nutritional status (BMI / U) and hemoglobin levels in adolescent girls.

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CAVA SMOOTHIE AS AN ADJUVANT IN IRON SUPPLEMENTATION CAN INCREASE HEMOGLOBIN LEVELS AND ERYTHROCYTE INDICES IN ANEMIC ADOLESCENT GIRLS

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ABSTRACT

Uncontrolled anemia leads to a decline in hemoglobin levels and erythrocyte blood indices, posing various health risks. To address this, a study aimed to assess the effectiveness of cava smoothies and iron supplementation in boosting hemoglobin levels and erythrocyte indices among anemic adolescent girls. The study involved 87 adolescent girls with moderate anemia, divided into three groups. The control group (C) received weekly iron tablets (60 mg), while two intervention groups (I1 and I2) were given the same iron tablets along with cava smoothies in increasing doses over 28 days. The results revealed significant improvements in hemoglobin levels. Group C showed an increase of 0.27 g/dL, while I1 and I2 demonstrated remarkable gains of 1.46 g/dL and 2.54 g/dL, respectively. Furthermore, erythrocyte indices (MCV, MCH, MCHC) exhibited positive changes. Group C saw increases of 0.35 fl, 0.34 pg, and 0.27 g/dL, I1 showed improvements of 0.69 fl, 0.61 pg, and 0.64 g/dL, and I2 experienced substantial enhancements of 1.97 fl, 1.61 pg, and 1.60 g/dL. In conclusion, the study confirmed that cava smoothies and iron supplementation effectively raise hemoglobin levels and improve erythrocyte indices in anemic adolescent girls, highlighting the potential benefits of this intervention for combating anemia.

Keywords: anemia, cava smoothie, hemoglobin, erythrocyte indices, iron supplementation

INTRODUCTION

Anemia is defined as a condition in which the hemoglobin levels are insufficient to meet the body's physiological needs (Miles & Richards, 2022). Anemia is associated with physical impairments, reduced quality of life, increased susceptibility to infections, elevated morbidity and mortality rates in women and children, decreased work productivity, cognitive impairments, and developmental disorders in preschool-age children and reproductive-aged women (Chaparro & Suchdev, 2019; Richards *et al.*, 2021). Individuals with anemia are more commonly found among adolescent girls, as adolescent girls are relatively ten times more likely to experience anemia compared to adolescent boys (Kemenkes RI, 2018). The prevalence of anemia in Southeast Asia ranges from 14.0% to 46.0%, while in Indonesia, it stands at 28.0%, making Indonesia the fifth-ranked country in Southeast Asia regarding anemia

prevalence (World Health Organization, 2016). Anemia due to iron deficiency, or Iron Deficiency Anemia (IDA), is an ongoing nutritional issue in Indonesia. Data from the Basic Health Research (Riskesdas) indicates that the prevalence of IDA among adolescents in Indonesia has increased from 37.0% in 2013 to 48.1% in 2018 (Martiasari *et al.*, 2022; Badan Penelitian dan Pengembangan Kesehatan, 2018).

The management of anemia in adolescent girls can be carried out through a government program known as the Iron Supplementation Tablets (IST) program (Yudina & Fayasari, 2020). Circular Letter from the Ministry of Health of the Republic of Indonesia Number HK.03.03/V/0595/2016 regarding the provision of IST to Adolescent Girls and Women of Reproductive Age stipulates that IST should be given at a rate of 1 tablet per week per year (≥ 52 tablets/year). The consumption rate of IST in Indonesia remains low,

with 98.6% of adolescent girls consuming fewer than 52 tablets per year and only 1.4% meeting the recommended intake (Nurcahyanti *et al.*, 2022). The reason adolescent girls do not consume iron-fortified instant drinks (IFID) is due to feelings of boredom or laziness, as well as the unpleasant taste and aroma of IFID. Some adolescent girls also experience side effects such as nausea and upper abdominal pain (Ningtyias *et al.*, 2020). Innovative supplementary beverages rich in iron and Vitamin C are required to increase hemoglobin levels and erythrocyte indices.

Several food items can be utilized to help increase hemoglobin levels and erythrocyte indices, such as *Cavendish bananas*, butter avocados, pure honey, and Sukkari dates. Bananas are a commonly found fruit in many countries, particularly in Asia (Garcı *et al.*, 2015). The iron and vitamin C content in *Cavendish bananas* is relatively high, with 2.6 mg and 55.1 mg per 100 grams, respectively, both of which can assist in enhancing hemoglobin levels and erythrocyte indices (Aryani *et al.*, 2022). *Cavendish bananas* contain 0.7 grams of protein, 0.1 grams of fat, and 19.8 grams of carbohydrates per 100 grams (Aryani *et al.*, 2022). Avocado fruit has the potential to aid in addressing anemia in adolescent girls, as 100 grams of avocado contains 0.6 mg of iron and 8.80 mg of vitamin C (Dreher & Davenport, 2013). Avocado also contains 0.4 g of protein, 2.0 g of fat, and 8.7 g of carbohydrates in every 100 grams (Viera *et al.*, 2022). Honey can increase hemoglobin levels and erythrocyte indices in the blood, as it contains 6.4 mg of iron and 10.7 mg of vitamin C every 100 grams (Islam *et al.*, 2017). Honey also contains 6.1 g of protein, 0.5 g of fat, and 55.3 g of carbohydrates per 100 grams (Mustafa *et al.*, 2023). Dates also possess the potential to assist in alleviating anemia in adolescent girls. This is caused by the fact that 100 grams of dates contain 10.4 milligrams of iron and 1.71 milligrams of vitamin C (Karajibani, 2019; Olabinjo *et al.*, 2022). Additionally, within the same 100-gram portion, dates provide 2.0 grams of protein, 3.6 grams of fat, and 82.6 grams of carbohydrates (Zar Pasha *et al.*, 2022).

The current popular beverage trend is the smoothie, which is primarily sold in fast-food

establishments (Šilha *et al.*, 2022). Smoothies are commonly consumed by children and adolescents (Fidler Mis *et al.*, 2017). Smoothies not only offer a wealth of nutritious and healthful ingredients but also stand out due to their distinctive and unique presentation, making them more appealing to consumers (Malau *et al.*, 2019). The preliminary examination conducted by the researcher determined that the composition of cava smoothie in 100 grams includes 4.1 grams of protein, 1.0 grams of fat, 30.6 grams of carbohydrates, 41.2 milligrams of vitamin C, and 12.5 milligrams of iron.

Many studies indicate that bananas, avocados, honey, and dates are local food sources with the potential to control anemia. The first study is being conducted by Rifiana & Hardiani (2021), which shows that the consumption of 100 grams of banana significantly increases hemoglobin levels over a 14-day period in anemic adolescent girls. Related research on the utilization of avocados is also being carried out by Feriyal (2017), showing that the consumption of 200 grams of avocado juice significantly increases hemoglobin levels over a 14-day period in anemic adolescent girls. Meanwhile, research on the potential of honey was conducted by Cholifah & Wulandari (2018), demonstrating that giving 3 tablespoons of honey per day for 14 days can significantly increase hemoglobin levels in anemic adolescent girls. Additionally, a study conducted by Susilawati (2022) indicates that the consumption of four dates over 7 days has a significant impact on increasing hemoglobin levels in anemic adolescent girls.

Although several researchers have conducted research on bananas, avocados, honey, and dates, a study on the combined effects of banana, avocado, honey, and dates in the form of a cava smoothie to improve hemoglobin levels and erythrocyte indices over a 28-day period, with the addition of iron supplementation, has never been undertaken. Therefore, this represents a novelty in research. Based on the description above, the researchers are interested in verifying whether a cava smoothie and iron supplementation can increase hemoglobin levels and erythrocyte indices in anemic adolescent girls.

METHOD

Data source and study design

This study was a quasi-experimental research with a pretest-posttest control group design. The study subjects consisted of adolescent girls with anemia, and the inclusion criteria were as follows: Adolescent girls with hemoglobin levels between 8.0-10.9 g/dL (experienced moderate anemia), aged 13-18 years, had regular menstruation occurring once a month (with cycles ranging from 21-35 days) for approximately 7 days, had a preference for bananas, avocados, honey, and dates, and did not suffer from chronic illnesses (such as intestinal parasites and malaria). Exclusion criteria included: Absence during data collection, illness during the study, and unwillingness to consume the cava smoothie. The research was conducted in five Islamic boarding schools in Metro City: Roudlotut Tholibin Islamic Boarding School (North Metro), Roudlatul Qur'an Islamic Boarding School (West Metro), Darul A'mal Islamic Boarding School (West Metro), Muhammadiyah At-Tanwir Islamic Boarding School (West Metro), and Daarul Ulya Islamic Boarding School (East Metro) in January-February 2023. The reason for selecting these five Islamic boarding schools as research locations was based on secondary data from the Metro City Health Office (year 2022), indicating that these boarding schools were in the top three areas with the highest prevalence of anemia in Metro City. From the population in these five boarding schools, a purposive sampling method was used to obtain a sample of 87 adolescent girls with moderate anemia.

The research subjects were then randomly divided into 3 groups, with each group consisting of a mix of individuals from various Islamic boarding schools. Each group comprised 29 adolescent girls, namely: Group C (Control Group), consisting of adolescent girls with anemia who were only given iron supplementation; Group I1 (Intervention Group 1), consisting of adolescent girls with anemia who were given iron supplementation (60 mg) and a cava smoothie at a dosage of 100 ml; and Group I2 (Intervention Group 2), consisting of adolescent girls with anemia who were given iron supplementation (60 mg) and a cava smoothie at a dosage of 200 ml. All adolescent girls with anemia

were provided with iron supplementation in the form of Blood-Boosting Tablets at a dosage of 60 mg once a week if they were not menstruating and 7 times a week if they were menstruating. Adolescent girls with anemia were also given Cava Smoothie in two dosages: 100 ml and 200 ml every 2 days. The compliance level of the respondents in consuming iron supplementation and Cava Smoothie was assessed through the completion of questionnaires/forms in which they recorded the timing of iron supplementation and Cava Smoothie consumption, accompanied by their signatures and the endorsement of the responsible individuals at the Islamic boarding schools.

Research tools and materials

The research tools used to prepare the cava smoothie included: a blender, plastic cups, disposable hand gloves, measuring spoons, a kitchen scale, a knife, standing pouches, a refrigerator, and an ice cooling box. The research tools used for blood sample collection included: disposable gloves, 3 ml injection syringes, 3 ml EDTA tubes, 70% alcohol swabs, adhesive bandages, and a tourniquet. Meanwhile, the instrument used for analyzing blood serum (hemoglobin levels and erythrocyte indices) was the Dirui BC 3600 Hematology Analyzer.

The ingredients used to make a cava smoothie include: ripe white *Cavendish bananas* and ripe buttery avocados, pure honey, and Sukkari dates. The bananas and avocados were sourced from plantations in Lampung Province. Ripe bananas were selected based on characteristics such as bright, evenly yellow skin extending from the middle to the tips, a rounded shape, and smooth and soft banana skin (picked at 3 months of fruit age). The ripe avocados were selected based on characteristics such as dark green to deep purple skin, a soft texture, easy separation of the fruit from the skin, and easy removal of the seed from the flesh (picked at 6-7 months of fruit age). Pure honey and Sukkari dates were obtained from the Tayyiba store in Surakarta City.

The materials for the examination of hemoglobin levels and erythrocyte indices include 3 ml of blood drawn from a vein, 70% alcohol, and EDTA. Reagents for the examination of

hemoglobin levels and erythrocyte indices consist of hematology reagents such as Lyse, Cleanser, Diluent, and roll paper.

Preparation of cava smoothie

The cava smoothie was made from the flesh of Cavendish bananas, buttery avocados, pure honey, and Sukkari dates. The process of making Cava Smoothie began with handwashing and cleaning of utensils and ingredients. Plastic gloves were then worn. Cavendish bananas and buttery avocados were peeled and sliced. Slices of Cavendish bananas (75 g) and buttery avocados (75 g) were placed into a standing pouch and stored in the freezer overnight at a temperature of -17°C. After one night, once the bananas and avocados had frozen, they were transferred into a blender container. Pure honey (25 ml) and Sukkari dates (25 g) were added to the blender. All the ingredients were blended until smooth and homogeneous. The smoothie was then poured into 100 ml and 200 ml plastic cups (according to the research dosage). To keep the cava smoothie cold, it was placed in an ice-cooling box. The cava smoothie was ready to be served to the research subjects.

The gives of iron supplements and cava smoothie.

Before being given iron supplementation and cava smoothie treatment, research subjects were instructed on the stages that would be conducted in the study and a commercial brand of deworming medication (albendazole). The deworming medication was given as a single dose of 400 mg and taken 21 days before the treatment began. Iron supplementation was achieved by providing commercial-brand iron supplement tablets (iron-fortified tablets) containing 60 mg of iron per tablet. Iron supplement tablets were given once a week to research subjects who were not menstruating, and for research subjects who were menstruating, they were given once a day for 7 consecutive days, and after menstruation, they were given once a week. Iron supplement tablets were provided to all groups, including the control group (C) and the intervention groups (I1 and I2). In addition to receiving iron supplementation, the intervention groups also received a cava

smoothie every 2 days. Group I1 received cava smoothie dose 1 (100 ml) every 2 days, while group I2 received dose 2 (200 ml) every 2 days. The treatment with iron supplements and a cava smoothie was carried out over a period of 28 days.

The examination of Hb levels and erythrocyte indices

The examination of Hb levels and erythrocyte indices to determine the effect of cava smoothie was conducted before and after the treatment, specifically 1 day before the treatment (H0) and after 28 days of treatment (H29). Blood collection and the examination of Hb levels and erythrocyte indices were performed at the Iringmulyo Public Health Center Laboratory, East Metro. Blood was drawn from the cubital vein using a 3 ml injection syringe, and then the blood was collected into a 3 ml EDTA tube (serum tube). Subsequently, the blood in the tube was diluted with Dirui BC 3600 reagent. The measurement of Hb levels and erythrocyte indices was performed using the impedance method, automatically calculating red blood cells (Isma et al., 2017). Hb levels are expressed in units of g/dL, while erythrocyte indices include MCV (mean corpuscular volume) expressed in units of fl, MCH (mean corpuscular hemoglobin) expressed in units of pg, and MCHC (mean corpuscular hemoglobin concentration) expressed in units of g/dL.

Data analysis

In this study, to determine the differences in Hb levels and erythrocyte indices before and after treatment within each group, data were analyzed using the Paired T-Test (for normally distributed data). However, for data with non-normal distribution, the Wilcoxon test was used for analysis. To determine the differences in the hemoglobin level and erythrocyte indices among the groups, the data were analyzed using the One-Way ANOVA test, followed by Post Hoc tests (for normally distributed data). However, for data with non-normal distribution, the Kruskal-Wallis test was used, followed by the Mann-Whitney test (Sumardiyono, 2020).

In this study, the significance level used is $\alpha=0.05$. The analysis results are considered

significant if the p-value is less than 0.05 ($p < 0.05$), and they are considered not significant if the p-value is greater than or equal to 0.05 ($p \geq 0.05$).

Ethical approval

All procedures conducted in this study have been approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Sebelas Maret Surakarta, with letter number 119/

UN27.06.11/KEP/EC/2022 dated September 26, 2022.

RESULTS AND DISCUSSION

Respondent Characteristics

Table 1 presents the characteristics of the respondents, including Age, Education, and Hb Levels.

Table 1. Subject Characteristics

Variable	Category	Group						p-value
		C		I1		I2		
		N	%	N	%	N	%	
Age	Early Adolescents (10-13 years)	1	3.4	3	10.3	3	10.3	0.399
	Middle Adolescents (14-17 years)	26	89.7	24	82.8	25	86.3	
	Late Adolescents (18-24 years)	2	6.9	2	6.9	1	3.4	
	Total	29	100.0	29	100.0	29	100.0	
Education	Junior High School (SMP/MTs)	12	41.4	21	72.4	17	58.6	0.057
	Senior High School (SMA/MA)	17	58.6	8	27.6	12	41.4	
	Total	29	100.0	29	100.0	29	100.0	
Hb Levels	Mild Anemia (11,0-11,9g/dL)	0	0.0	0	0.0	0	0.0	0.332
	Moderate Anemia (8,0-10,9g/dL)	29	9.5	29	10.3	29	10.2	
	Severe Anemia (<8,0g/dL)	0	0.0	0	0.0	0	0.0	
	Total	29	100.0	29	100.0	29	100.0	

Note: C = Control Group (given iron supplementation 60 mg/week); I1 = Intervention Group 1 (given iron supplementation 60 mg/week and cava smoothie 100 ml/every 2 days); I2 = Intervention Group 2 (given iron supplementation 60 mg/week and cava smoothie 200 ml/every 2 days).

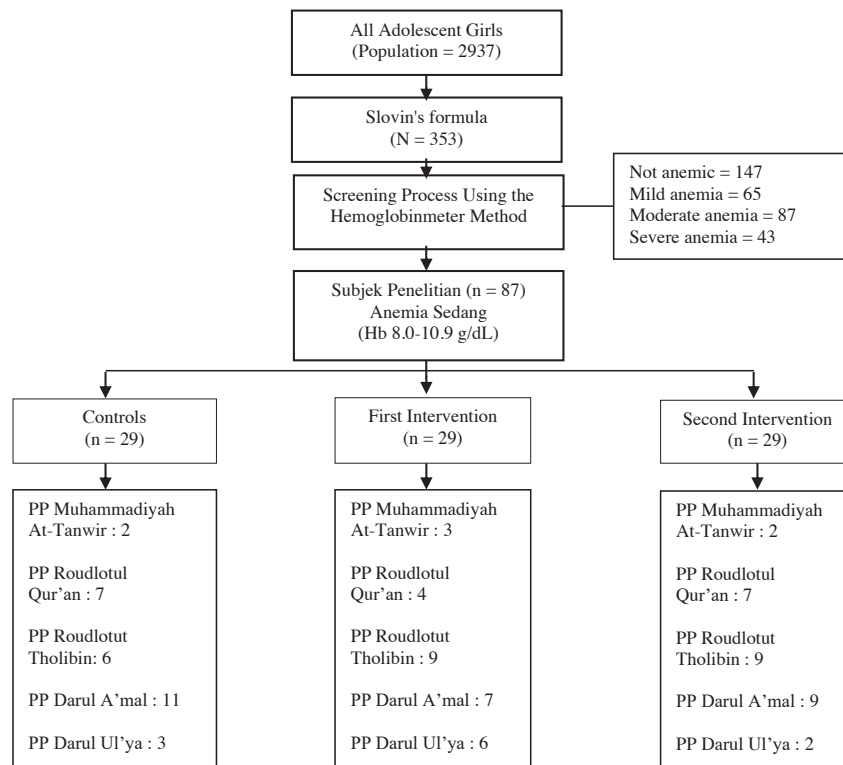


Figure 1. Research Subject Allocation Flowchart

Note: PP = Islamic Boarding School

Figure 1 illustrates that to divide the research subjects, it can begin by collecting all adolescent girls from 5 Islamic boarding schools in Metro City, totaling 2937 adolescent girls. Then, using Slovin’s formula, 353 adolescent girls were selected. From these 353 adolescent girls, a screening process was conducted using the hemoglobinometer method, resulting in 147 non-anemic adolescent girls, 65 with mild anemia, 87 with moderate anemia, and 43 with severe anemia.

The researchers selected adolescent girls with moderate anemia because the intervention used was appropriate for addressing it, which is the provision of iron supplementation and cava smoothie. The researchers did not choose adolescent girls with mild anemia because mild anemia can be addressed simply by consuming iron-rich foods, whereas severe anemia cannot be addressed with just iron supplementation or cava smoothie alone. In other words, severe anemia requires more serious treatment compared to mild and moderate anemia.

The 87 research subjects, who were adolescent girls with anemia, were divided into 3 groups randomly, with each group consisting of 29 adolescent girls with anemia. These 29 adolescent girls with anemia were a combination of individuals from 5 Islamic boarding schools in Metro City.

Table 1 shows that the total research subjects were 87 respondents, with the majority of adolescent girls falling into the mid-teenage age group (14-17 years old), totaling 72 individuals. The highest educational level among the adolescent girls was junior high school (SMP/MTs), with 50 individuals. All adolescent girls had hemoglobin (Hb) levels categorized as moderate anemia, ranging from 8.0-10.9 g/dL. Based on the results of statistical tests, it was found that there was no significant difference among the three groups in terms of age, education, and Hb levels, indicating homogeneity among the research subjects (p -value > 0.05).

Giving Cava Smoothie and Iron Supplementation Increases Hemoglobin Levels

Table 2. Mean Hemoglobin Levels on Day 0 and Day 28, and the Difference in Each Group

Group	Mean ± SD (g/dL)			Kruskal-Wallis Test
	C	I1	I2	
Day 0	10.14 ± 0.60	10.05 ± 0.68	9.90 ± 0.78	0.653
Day 28	10.41 ± 0.29	11.51 ± 0.84	12.44 ± 0.79	0.001*
▲ (g/dL)	0.27 ± 0.57 ^a	1.46 ± 0.96 ^b	2.54 ± 1.24 ^c	0.001*
Wilcoxon Test	0.140	0.001*	0.001*	

Note: C = Control Group (given iron supplementation 60 mg/week); I1 = Intervention Group 1 (given iron supplementation 60 mg/week and cava smoothie 100 ml/every 2 days); I2 = Intervention Group 2 (given iron supplementation 60 mg/week and cava smoothie 200 ml/every 2 days); a, b, c = Significantly different in Mann-Whitney Test; * = Significantly different (<0.05).

Figure 2. Bar Chart of Mean Hemoglobin Levels on Day 0 and Day 28 in Each Group

Legend: C = Control Group; I1 = Intervention Group 1; I2 = Intervention Group 2

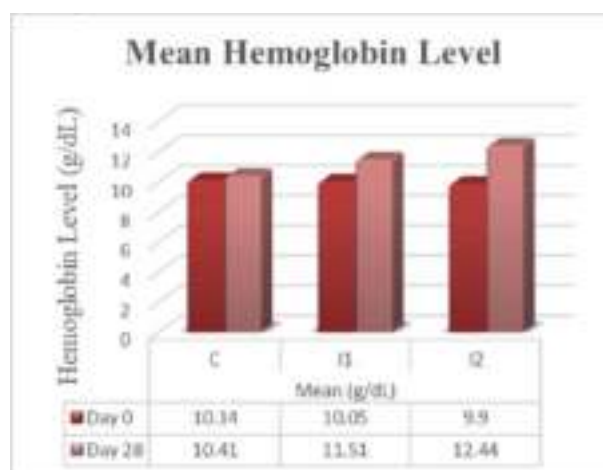


Table 2 and Figure 2 demonstrate that between day 0 and day 28, both intervention group 1 and intervention group 2 experienced a significant increase in hemoglobin levels, while the control group showed an increase in hemoglobin levels, but it is not statistically significant. The most substantial increase in hemoglobin levels is observed in intervention group 2, followed by intervention group 1, and the smallest increase is in the control group. The nonsignificant increase in hemoglobin levels in the control group suggests that providing iron supplementation alone may not lead to a significant improvement in hemoglobin levels in adolescent girls experiencing moderate anemia. This finding is inconsistent with the study by Jalambo *et al.* (2018), which indicated that consuming iron supplements could increase hemoglobin levels in the blood. This is because iron deficiency conditions can stimulate increased iron absorption in anemic subjects. Firstly, the absorbed iron can be utilized to normalize hemoglobin levels, ensuring that the supply of oxygen to tissues/cells is sufficient (Susanti *et al.*, 2016). This means that providing iron supplementation alone may not effectively contribute to tissue/cell oxygenation.

The statistical test results in this study indicate that hemoglobin levels in intervention group 1 and intervention group 2 are significantly higher than those in the control group. This implies that cava smoothies, made from bananas, avocados, honey, and dates, when combined with iron supplementation, can significantly increase hemoglobin levels. As it is known from laboratory test results of its nutritional content, cava smoothie contains iron and vitamin C, among other nutrients. The presence of iron content can increase hemoglobin levels because iron is a component of the formation of hemoglobin (Abbaspour *et al.*, 2014). Additionally, the presence of vitamin C content will also increase hemoglobin levels because vitamin C aids in the absorption of iron from food/drinks, allowing it to be processed into red blood cells once again (Chavan *et al.*, 2021).

The statistical test results also indicate that the increase in hemoglobin levels in intervention group 2 is significantly higher compared to intervention group 1. Furthermore, in group I2, after receiving iron supplementation and cava smoothie, the

mean hemoglobin level shows a normal value of 12.2 ± 0.6 mg/dL, while in group I1, the mean hemoglobin level is still below normal. This means that an increased dosage of cava smoothie can provide a more optimal increase in hemoglobin levels. The results of this study align with research conducted by Tuju *et al.* (2019), which showed that a combination of 200 g of bananas and 60 mg of iron supplementation for 7 days could significantly increase hemoglobin levels compared to iron supplementation alone. Another study conducted by Utami (2020), also indicates that the combination of 100 g of avocado and 60 mg of iron supplementation for 7 days can result in a higher increase in hemoglobin levels compared to iron supplementation alone. Damayanti *et al.* (2021) also researched the combination of 1 tablespoon of honey and 60 mg of iron supplementation for 14 days, resulting in a higher increase in hemoglobin levels than 60 mg of iron supplementation alone. Additionally, the study by Novadela & Imron (2015) showed that the combination of 2 dates and 60 mg of iron supplementation for 21 days could lead to a higher increase in hemoglobin levels compared to 60 mg of iron supplementation alone.

Bananas contain vitamin C, vitamin B1, energy, fat, carbohydrates, calcium, phosphorus, iron, and water (Ruspita *et al.*, 2022). The iron content in bananas can stimulate hemoglobin production in the blood (Adethia & Sukarni, 2022). The vitamin C content in bananas can accelerate the absorption of iron and help in the treatment of anemia patients (Mahardika & Zuraida, 2016). Consuming avocados, which are rich in iron and vitamin C, is very beneficial for the growth of red blood cells in the body and can help prevent and treat anemia, thus addressing complications resulting from anemia (Kiswari, 2018). Honey is a sweet-tasting herbal substance produced by honeybees from the nectar of flowers or liquids derived from plant matter, which is collected, modified, and combined into a positive compound by bees. The mineral magnesium content in honey is similar to the magnesium content in human blood serum. Similarly, the iron content in honey can increase the number of red blood cells in human blood and enhance hemoglobin levels (Panjaitan, 2018). Dates are a high-energy

source of nutrition with an ideal composition. They contain carbohydrates, tryptophan, omega-3, vitamin C, vitamin B6, Ca2, Zn, and Mg, and are rich in fiber. Additionally, they contain potassium, manganese, phosphorus, iron, sulfur, calcium, and magnesium.

The increase in hemoglobin levels occurs after the consumption of dates (As *et al.*, 2021).

Giving Cava Smoothie and iron supplementation increases red blood cell indices (MCV, MCH, MCHC).

Table 3. Mean MCV (Mean Corpuscular Volume) on Day 0 and Day 28, and the Difference in Each Group

Group	Mean ± SD (fL)			Kruskal-Wallis Test
	C	I1	I2	
Day 0	79.05 ± 3.25	78.97 ± 6.47	78.82 ± 1.99	0.127
Day 28	79.40 ± 3.01	79.67 ± 6.50	80.79 ± 1.67	0.010*
▲ (fL)	0.35 ± 0.64 ^a	0.69 ± 2.12 ^b	1.97 ± 1.19 ^c	0.001*
Wilcoxon Test	0.120	0.001*	0.001*	

Note: C = Control Group (given iron supplementation 60 mg/week); I1 = Intervention Group 1 (given iron supplementation 60 mg/week and cava smoothie 100 ml/every 2 days); I2 = Intervention Group 2 (given iron supplementation 60 mg/week and cava smoothie 200 ml/every 2 days); a, b, c = Significantly different in Mann-Whitney Test; * = Significantly different (<0.05).

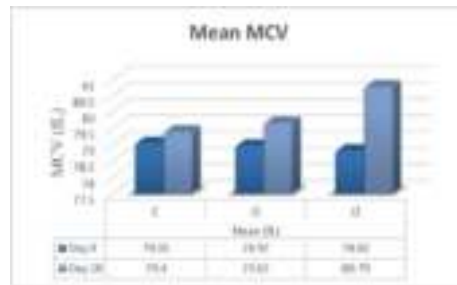


Figure 3. Bar Chart of Mean MCV on Day 0 and Day 28 in Each Group
 Legend: C = Control Group; I1 = Intervention Group 1; I2 = Intervention Group 2.

Table 4. Mean MCH (Mean Corpuscular Hemoglobin) on Day 0 and Day 28 and the Difference in Each Group

Group	Mean ± SD (pg)			Kruskal-Wallis Test
	C	I1	I2	
Day 0	25.13 ± 0.69	25.05 ± 0.68	24.90 ± 0.77	0.616
Day 28	25.47 ± 0.39	25.66 ± 0.58	26.51 ± 0.84	0.001*
▲ (pg)	0.34 ± 0.62 ^a	0.61 ± 0.69 ^b	1.61 ± 0.99 ^c	0.001*
Wilcoxon Test	0.050	0.010*	0.010*	

Note: C = Control group (given iron supplementation 60 mg/week); I1 = Intervention group 1 (given iron supplementation 60 mg/week and cava smoothie 100 ml/every 2 days); I2 = Intervention group 2 (given iron supplementation 60 mg/week and cava smoothie 200 ml/every 2 days). a, b, c = Significantly different in Post Hoc Test, * = Significantly different (<0.05).



Figure 4. Bar Chart of Mean MCH on Day 0 and Day 28 in Each Group
 Legend: C = Control Group; I1 = Intervention Group 1; I2 = Intervention Group 2

Table 5. Mean MCHC (Mean Corpuscular Hemoglobin Concentration) on Day 0 and Day 28 and the Difference in Each Group

Group	Mean \pm SD (g/dL)			Kruskal-Wallis Test
	C	I1	I2	
Day 0	32.13 \pm 0.59	32.00 \pm 0.68	31.88 \pm 0.77	0.562
Day 28	32.40 \pm 0.31	32.64 \pm 0.60	33.48 \pm 0.83	0.001*
▲ (%)	0.27 \pm 0.55 ^a	0.64 \pm 0.66 ^b	1.60 \pm 0.99 ^c	0.001*
Wilcoxon Test	0.090	0.010*	0.010*	

Note: C = Control Group (given iron supplementation 60 mg/week); I1 = Intervention Group 1 (given iron supplementation 60 mg/week and cava smoothie 100 ml/every 2 days); I2 = Intervention Group 2 (given iron supplementation 60 mg/week and cava smoothie 200 ml/every 2 days); a, b, c = Significantly different in Mann-Whitney test; * = Significantly different (<0.05).

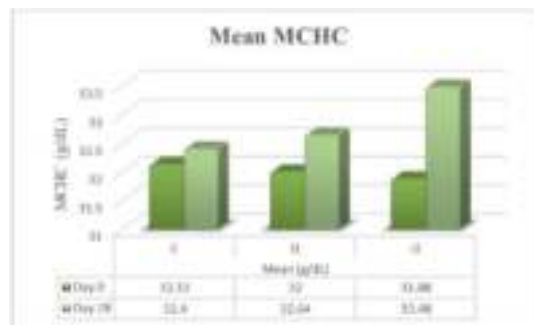


Figure 5. Bar Chart of Mean MCHC on Day 0 and Day 28 in Each Group
Legend: C = Control Group; I1 = Intervention Group 1; I2 = Intervention Group 2.

Tables 3, 4, and 5, as well as Figures 3, 4, and 5, indicate that between day 0 and day 28, both intervention groups 1 and 2 experienced a significant increase in MCV, MCH, and MCHC, while the control group showed an increase that was not statistically significant. The increase in MCV, MCH, and MCHC was most pronounced in intervention group 2, followed by intervention group 1, and was smallest in the control group. The non-significant increase in MCV, MCH, and MCHC in the control group indicates that iron supplementation alone may not significantly improve MCV, MCH, and MCHC in adolescent girls with moderate anemia. This finding is inconsistent with the study conducted by Zhang *et al.* (2020), which found that iron supplementation can significantly increase MCV in the blood. Dissimilar results were also found in a study conducted by Wahyuni (2021), which revealed that consuming iron supplements can significantly increase MCH and MCHC in the blood. The statistical analysis results in this study indicate that MCV, MCH, and MCHC in intervention group 1 and intervention group 2 are significantly higher than in the control group. This means that cava smoothies made from bananas, avocados,

honey, and dates, when combined with iron supplementation, can significantly increase MCV, MCH, and MCHC levels. As known, based on the laboratory analysis of nutritional content, cava smoothie contains, among other things, iron and vitamin C. Iron and vitamin C are best consumed together. Vitamin C assists the body in absorbing non-heme iron by binding to it and helping it flow into the intestines. When vitamin C binds with non-heme iron, it enhances its stability and solubility. This allows the body to more easily absorb iron through the intestinal mucosa (Piskin *et al.*, 2022).

The statistical results also indicate that the increase in MCV, MCH, and MCHC in intervention group 2 is significantly higher compared to intervention group 1. This means that an increase in the dosage of cava smoothie can provide a more optimal effect on MCV, MCH, and MCHC. In this study, cava smoothie was administered over a period of 28 days with a graded dosage, namely 100 ml every 2 days and 200 ml every 2 days. MCV, or Mean Corpuscular Volume, serves to measure the average size of red blood cells, and if MCV is low, it indicates that the red blood cells are very small. MCH and MCV are directly related;

if MCV increases, MCH will also increase, and if MCV is low, MCH will be low as well (Peng *et al.*, 2021). MCH is directly proportional to MCHC; if MCH increases, then MCHC will also increase. The increase in MCHC occurs due to the improving condition of iron-deficiency anemia (Fitriany & Saputri, 2018).

CONCLUSION AND RECOMMENDATIONS

It can be concluded that giving Cava Smoothie and iron supplementation is effective in increasing hemoglobin levels and red blood cell indices (MCV, MCH, and MCHC) in anemic adolescent girls ($p < 0.05$). However, further research is needed to examine parameters for anemia beyond hemoglobin levels and red blood cell indices, such as hepcidin and serum ferritin.

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DAFTAR PUSTAKA

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NUTRACEUTICAL POTENTIAL OF ENCAPSULATED PURPLE OKRA (*Abelmoschus esculentus* L. Moench) EXTRACT

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ABSTRACT

The accumulation of free radical production impacts on the development of degenerative diseases which are the highest cause of morbidity and mortality in the world. Biofortification of purple okra in the form of encapsulated extract has the potential as a nutraceutical through the role of antioxidants. This study aimed to analyze the potential of encapsulated purple okra extract as a nutraceutical by determining physical-chemical characteristics, microbial and heavy metals contamination, antioxidant capacity, total flavonoids, and quercetin. This was a laboratory experimental study on purple okra which was extracted using the maceration method. The results of physical-chemical characteristics showed that purple okra extract has the form of dry powder, greenish-brown color, odor characteristic of okra extract (caramel-like), and sour taste with yield of 4%, pH of 4.8, undetectable solvent residue, water content of 13.5%, total ash content of 10.4%, and disintegration time of 1.25 minutes. The results of microbial contamination showed a total plate count of 3.1×10^2 CFU g⁻¹, yeast and mold count, *E. coli*, *S. aureus* negative CFU g⁻¹, and *Salmonella* spp negative CFU 10g⁻¹. The results of heavy metal contamination showed that As, Pb, Cd, and Hg were not detected every mg Kg⁻¹. The results of antioxidant capacity, total flavonoids, and suspected quercetin derivatives showed a value of 84.88%, 81.32 mg QE g⁻¹, and 4.91 mg g⁻¹. These bioactive components act as free radical scavengers in helping to prevent chain reactions. Encapsulated purple okra extract has shown its potential as a nutraceutical that helps prevent degenerative diseases.

Keywords: antioxidant, encapsulated, nutraceutical, purple okra extract

INTRODUCTION

The prevalence of degenerative diseases is estimated to continue to increase and become the main cause of death globally (Ramesh & Kosalram, 2023). The accumulation of free radicals has an impact on the development of various degenerative diseases through various mechanisms (Sharifi-Rad et al., 2020). Free radicals form reactive oxygen species (ROS) which have one or more unpaired electrons. When the production of free radicals exceeds the limit of protective capabilities, it will initiate an autocatalytic reaction to induce damage to the main components of cells, namely protein, lipid, and deoxyribonucleic acid (DNA) (Burgos-Morón et al., 2019). This condition is the beginning of oxidative stress due to an imbalance between free radical production and natural radical scavengers (Salsabila et al., 2022).

Exogenous antioxidant sources from food or parts of food are needed when the production of endogenous antioxidants as natural radical

scavengers is unable to compensate for the increase in free radicals (El-Masry & Mahmoud, 2021). The availability of exogenous antioxidants can restrain the use of endogenous antioxidants, thereby synergistically increasing defense against oxidative stress (Moussa et al., 2019). Antioxidants work by giving one electron to oxidant compounds so that their activity can be inhibited (Hurrell & Hsu, 2017). Okra is starting to develop in Asia including Indonesia which has been identified as having antioxidant activity with considerable market potential (Bawa & Badrie, 2016). Biofortification of purple okra (*Abelmoschus esculentus* L. Moench) developed by the Center for Tropical Horticulture Studies, IPB University has bioactive components in the form of flavonoid and quercetin which are superior to green okra. Purple okra is the result of developing superior seeds from okra varieties (Anjani, 2018).

The bioactive components in purple okra can be utilized in the form of encapsulated extract.

The encapsulated form facilitates use, controls the release of its active substances at the right target, and protects against external factors to maintain functional stability during storage (Nining et al., 2017; Suwaris & Saputra, 2020). Purple okra is extracted using a maceration method to extract bioactive components so that it has a higher content than the fresh form of okra (Achmad, 2022). The vacuum pan evaporator technology is used to dry the extract with the consideration that reducing pressure can reduce the boiling point so that the temperature is relatively lower and the time is relatively shorter to maintain the bioactive components (Syakdani et al., 2019).

The potential of encapsulated purple okra extract as a nutraceutical was identified through the role of antioxidants as free radical scavengers in helping to prevent chain reactions thereby preventing further damage to cell components (Yunanto et al., 2009). Research by Elkhalifa et al. (2021) regarding the potential of okra as a nutraceutical for health applications, showed its benefits as an antioxidant, antidiabetic, antihyperlipidemic, antiproliferative, and anticancer. Nutraceuticals, a combined term between nutrition and pharmaceuticals, are food or parts of food that provide added value to improve and enhance the body's physiological functions (Siddiqui & Moghadasian, 2020). This research aims to analyze the potential of encapsulated purple okra extract as a nutraceutical by determining physical-chemical characteristics, microbial and heavy metal contamination, as well as bioactive components that help prevent and reduce the consequences of degenerative diseases.

METHODS

This research used a laboratory experimental design which began with the production of encapsulated purple okra extract at the Pilot Plant, Department of Food Science and Technology, IPB University. The research continued with the analysis of physical-chemical characteristics, microbial and heavy metal contamination, and bioactive components (antioxidant capacity, total flavonoids, and quercetin) at Saraswanti Indo Genetech Laboratory, Bogor and Analysis of Nutrients and Biochemistry Laboratory,

Department of Community Nutrition, IPB University.

Encapsulated Purple Okra Extract Production

Purple okra is the result of the biofortification of okra varieties located at the Leuwikopo Experimental Garden which was developed by Prof. Dr. Muhamad Syukur, S.P., M.Sc. from the Center for Tropical Horticulture Studies, IPB University. Biofortification is carried out by including nutritional elements to produce okra varieties with superior bioactive components.

The production of encapsulated purple okra extract using a modification of research by Fan et al. (2014) and Achmad (2022) is presented in Figure 1. Fresh purple okra was blanched at 100 °C for 1 minute and mashed for 2 minutes. The extraction process was carried out using the maceration method at room temperature for 3×24 hours. Extraction results were dried using a vacuum pan evaporator at 60 °C for 30 minutes. The dry extract was powdered for 10 seconds and packed into capsules.

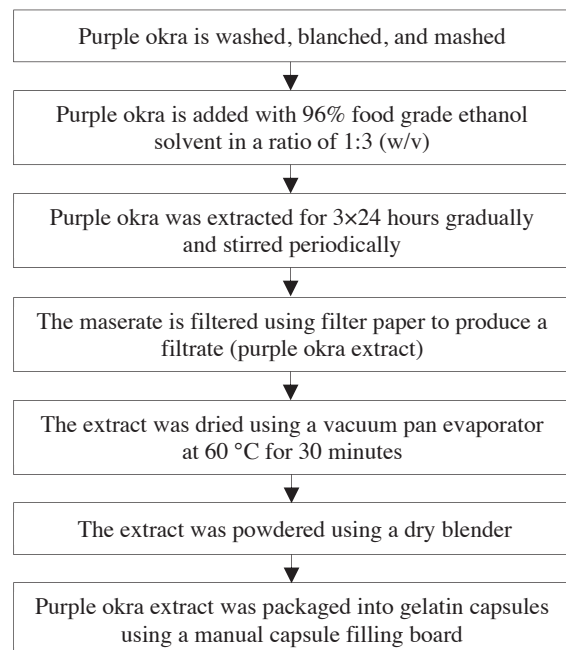


Figure 1. Production Process of Encapsulated Purple Okra Extract

Physical-Chemical Characteristics Analysis

Analysis of the physical-chemical characteristics of purple okra extract in the form

of organoleptic observations was carried out descriptively regarding texture, taste, odor, and color (BPOM, 2019). Yield analysis was carried out by the weighing method using an analytical balance (Depkes, 2000). pH analysis was carried out by the electrometric method using a pH meter (Vernanda et al., 2019).

Solvent residual analysis was carried out by the chromatographic method using gas chromatography according to the United States Pharmacopeia (USP) (2020a). Water content analysis was carried out by gravimetric method using an oven according to SNI 01-2891-1992 5.1 (BSN, 1992a). Total ash content analysis was carried out by the gravimetric method using a furnace according to SNI 01-2891-1992 6.1 (BSN, 1992b). Disintegration time analysis was carried out using a disintegration tester on encapsulated purple okra extract according to the Indonesian Pharmacopeia VI (Kemenkes, 2020).

Microbial Contamination Analysis

Analysis of microbial contamination in the form of total plate count (TPC), yeast mold count (YMC), and the specific microorganism *Escherichia coli* (*E. coli*) was carried out by the microorganism enumeration method using the pour plate technique which was counted using a colony counter according to SNI ISO 4833-1:2015 (BSN, 2015) for TPC, USP (2020b; 2020d) for YMC, and SNI ISO 16649-2:2016 (BSN, 2016) for *E. coli*. *Salmonella spp* analysis was carried out qualitatively using the inoculation method which was confirmed by biochemical test and serological test according to USP (2020b; 2020c). Analysis of *Staphylococcus aureus* (*S. aureus*) was carried out qualitatively using the inoculation method which was confirmed by the staining test and coagulation test according to USP (2020b; 2020c).

Heavy Metal Contamination Analysis

Analysis of heavy metal contamination such as arsenic (As) and lead (Pb) was carried out by the absorbance method using an inductively coupled plasma-mass spectrometer (ICP-MS) according to AOAC 2011.19 (AOAC, 2014), AOAC 2015.01 (AOAC, 2015), and Creed et al. (1994). Analysis of cadmium (Cd) and mercury (Hg) was carried

out by the absorbance method using inductively coupled plasma-optical emission spectrometry (ICP-OES) according to AOAC 2011.14 (AOAC, 2011), AOAC 2013.06 (AOAC, 2013), and Gomez et al. (2007).

Antioxidant Capacity Analysis

Antioxidant capacity analysis was carried out by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method using a microplate reader based on a modification of research by Molyneux (2004) and Khan et al. (2022). A sample of 0.01 g was dissolved with ethanol p.a in a 10 mL volumetric flask. Vitamin C standard of 0.1 g was dissolved with distilled water in a 10 mL volumetric flask. Vitamin C standard solution is diluted at a concentration of 10.000-0.001 ppm, while the sample solution is diluted at a concentration of 1000-31.25 ppm. A total of 150 μ L of each sample and the standard solution was added to 150 μ L of 0.2 mM DPPH solution and then homogenized. The solution was incubated for 30 minutes then the absorbance was measured at a wavelength of 515 nm and the antioxidant capacity was stated in %inhibition.

Total Flavonoids Analysis

Analysis of total flavonoids was carried out by the aluminum chloride (AlCl_3) method using a microplate reader based on a modification of research by Pascal et al. (2018). A sample of 0.01 g was dissolved with ethanol p.a in a 10 mL volumetric flask. Quercetin standard of 0.01 g was dissolved with ethanol p.a in a 10 mL volumetric flask. Quercetin standard solution is diluted at a concentration of 500-15.625 ppm. A total of 500 μ L of each sample and the standard solution was added 30 μ L of 5% NaNO_2 then homogenized and incubated for five minutes. A total of 30 μ L of 10% AlCl_3 solution was added then homogenized and incubated for one minute. A total of 200 μ L of 5% NaOH solution and 240 μ L of distilled water were added and then homogenized. Absorbance was measured at a wavelength of 492 nm. Total flavonoids are calculated through the equation $y = 0,0016x + 0,025$ and stated in mg quercetin equivalent (QE) g^{-1} .

Quercetin Analysis

Quercetin analysis was carried out by a chromatographic method using high-performance liquid chromatography (HPLC) based on a modification of research by Seo et al. (2021). A sample of 0.1 g was dissolved with HPLC-grade methanol in a 10 mL volumetric flask. The sample solution was filtered using a 0.45 µm nylon syringe filter. Quercetin standard of 0.0025 g was dissolved with the same solvent in a 10 mL volumetric flask. A total of 60 µl of sample filtrate was injected into the HPLC system. A low-pressure gradient system with methanol and water as mobile phases was used at ratios of 100:0 (3 minutes), 95:5 (3 minutes), and 90:10 (3 minutes) at a wavelength of 370 nm.

RESULTS AND DISCUSSION

Physical-Chemical Characteristics

The results of the physical-chemical characteristics analysis of encapsulated purple okra extract based on BPOM standard (2019) in encapsulated powder form, Depkes standard (2008), and Depkes standard (2000) in powder extract form are presented in Table 1. Organoleptic observation aims to provide a simple objective introduction by the researchers which is described in the form of dry powder, greenish-brown color, odor characteristic of okra extract (caramel-like), and sour taste. The results of the yield analysis of 4.0% show a different value compared to the standard yield according to Depkes (2000) of $\geq 7.2\%$. The yield provides an estimate of the bioactive components that can be extracted from the extraction process.

The results of the pH analysis show a value of 4.8. This is different from okra flour which is dried using a cabinet dryer which has a pH value of 6.35 (Fauza et al., 2019). The extraction process can lower the pH and lower pH values contain higher antioxidant activity (Rifkowitz & Wardanu, 2016). At low pH, the density of hydrogen increases which suppresses the release of hydrogen to scavenge free radicals (Fathinatullabibah et al., 2014). The residual solvent content after evaporation can be determined through residual solvent analysis to ensure the safety of the extract for consumption. The results show that solvent

Table 1. Physical-Chemical Characteristics of Encapsulated Purple Okra Extract

Parameter	Unit	Purple Okra Extract	Standard
Organoleptic			
Texture		dry powder	-
Color		greenish-brown	-
Odor		okra extract	-
Taste		sour	-
Yield	%	4.0	$\geq 7.2^1$
pH		4.8	-
Solvent residue	ppm	not detected	Max. 10.000 ²
Water content	%	13.5	$\leq 10^2$
Total ash content	%	10.4	$\leq 16.6^3$
Disintegration time	minute	1.25	$\leq 30^2$

Source: ¹Depkes (2000). ²BPOM (2019). ³Depkes (2008).

residue is not detected according to BPOM (2019). The vacuum pan evaporator technology is used to dry the extract which can evaporate the solvent at low pressure so that the temperature is relatively lower and the time is relatively shorter to maintain the bioactive components (Syakdani et al., 2019).

Furthermore, the results show a water content of 13.5% which is different from the standard water content according to BPOM (2019) of $\leq 10\%$. The presence of water content because the purple okra extract is hygroscopic which is able to absorb air humidity or water at normal temperature and normal pressure. Total ash content analysis provides an overview of the inorganic or mineral compounds remaining after the ashing process (Hidayati et al., 2018). The results show a total ash content of 10.4% which is different from the standard total ash content according to BPOM (2019) of $\leq 16.6\%$. The lower ash content indicates an optimal extraction process due to the low mineral residue (Khirzin et al., 2019). This is related to the use of solvents that attract more organic compounds than inorganic compounds.

The encapsulated form has an effect if it is first broken down into smaller particles so that it can be absorbed into the digestive tract. Encapsulated purple okra extract requires a disintegration time of 1 minute 15 seconds which shows a value in accordance with BPOM (2019), namely ≤ 30 minutes. The harder the capsule material, the

smaller the porosity, so it becomes more difficult to penetrate and absorb water into the capsule, which affects disintegration time (Sugiyanto et al., 2017).

Microbial and Heavy Metal Contamination

The results of microbial and heavy metal contamination analysis of encapsulated purple okra extract based on BPOM (2019) standards in encapsulated powder form are presented in Table 2. Microbial contamination analysis can provide assurance that microbial contamination does not exceed the specified limits to ensure the safety of the extract for consumption. The presence of microbes can affect the stability during storage and the safety of the extract. The result of TPC analysis shows a value of 3.1×10^2 colony-forming units (CFU) g^{-1} according to BPOM (2019) of $\leq 2 \times 10^4$ CFU g^{-1} . In addition, YMC analysis as well as specific microorganisms such as *E. coli*, *Salmonella spp*, and *S. aureus* showed negative results. These results are in accordance with BPOM (2019), namely $\leq 2 \times 10^2$ CFU g^{-1} for YMC, negative g^{-1} for *E. coli* and *S. aureus*, and negative $10g^{-1}$ for *Salmonella spp*. Research on the potential of okra as a nutraceutical shows its benefit as an antimicrobial that can inhibit bacterial growth (Syukri et al., 2020; Elkhalfifa et al., 2021).

Table 2. Microbial and Heavy Metal Contamination of Encapsulated Purple Okra Extract

Parameter	Unit	Purple Okra Extract	Standard ¹
Microbial contamination			
TPC	CFU g^{-1}	3.1×10^2	$\leq 2 \times 10^4$
YMC	CFU g^{-1}	negative	$\leq 2 \times 10^2$
<i>E. coli</i>	CFU g^{-1}	negative	negative
<i>Salmonella spp</i>	CFU $10g^{-1}$	negative	negative
<i>S. aureus</i>	CFU g^{-1}	negative	negative
Heavy metal contamination			
Arsenic	mg Kg^{-1}	not detected	≤ 5
Lead	mg Kg^{-1}	not detected	10
Cadmium	mg Kg^{-1}	not detected	≤ 0.3
Mercury	mg Kg^{-1}	not detected	≤ 0.5

Source: ¹BPOM (2019)

Heavy metal contamination analysis can provide assurance that heavy metal contamination does not exceed the specified limits that cause toxicity (Depkes 2000). The results show that heavy metal contamination such as As, Pb, Cd, and Hg are not detected according to BPOM (2019) in units of mg Kg^{-1} , namely ≤ 5 for As, 10 for Pb, ≤ 0.3 for Cd, and ≤ 0.5 for Hg. Heavy metal contamination can occur during the production of purple okra extract. In addition, industrial waste or agricultural activities such as the geological condition of the land where cultivation is carried out and the fertilizer used also affect contamination (Wijianto et al., 2022). Heavy metal contamination provides negative effects which are mostly mediated through increased production of excess ROS causing oxidative damage to various body's physiological systems (Awoke et al., 2020).

Antioxidant Capacity

The ability of antioxidant compounds in okra extract to inhibit free radicals is known as antioxidant capacity (%inhibition). The results of antioxidant capacity analysis showed that the %inhibition of purple okra extract was 84.88% (Table 3). The results of this study are different from the results of research by Zainuddin et al. (2022) on the ethanol extract of green okra which has an inhibition of 81.9%. The difference occurs because purple okra is the result of biofortification which has superior bioactive components compared to green okra (Anjani et al., 2018). Furthermore, the difference can also occur because the previous study used dry okra which was extracted and concentrated using a rotary evaporator at <50 °C to obtain a thick extract. In contrast to this study, which used fresh okra before the extraction process and vacuum pan evaporator technology to dry the extract. This technology works at a pressure of 65 cmHg so that the boiling point of the solvent can be lowered and the time required is relatively shorter (Syakdani et al., 2019). Heat treatment and light exposure for a certain time affect antioxidant compound that has thermolabile properties by triggering pre-oxidation (Rifkowitz & Wardanu, 2016).

Table 3. Antioxidant Capacity, Total Flavonoids, and Quercetin of Encapsulated Purple Okra Extract

Parameter	Unit	Purple Okra Extract
Antioxidant capacity	%	84.88
Total flavonoids	mg QE g ⁻¹	81.32
Quercetin	mg g ⁻¹	4.91

This is different from research by Anjani (2018) on green okra and purple okra in extract form which has an inhibition of 19.28% and 23.34%. Apart from being caused by differences in varieties and forms of dried okra before extraction, the difference in inhibition was also caused by the solvents used. In the previous study, okra was extracted using methanol, while in this study okra was extracted using ethanol. Research shows that the use of ethanol as a solvent shows higher amounts of bioactive components (Padmawati et al., 2020). Antioxidant compounds such as phenol are polar so using ethanol as a solvent is appropriate. Another study showed a different inhibition of 76.28% in okra fruit extract compared to okra leaf extract of 62.12% (Faisal & Handayani, 2019). This is because the fruit contains seeds and mucilages which are known to contain more antioxidant compounds, especially quercetin derivatives in the form of isoquercitrin (Chaemsawang et al., 2019).

Encapsulated purple okra extract with antioxidant content has the potential as a nutraceutical that can increase the capacity to withstand oxidative stress. Antioxidants can prevent, inhibit, eliminate, or repair oxidative damage to target molecules that occur as a result of chemical reactions involving free radicals (Banjarnahor & Artanti, 2014). Several mechanisms of antioxidant action include inhibition of enzymes involving the formation of ROS, termination of radical chain reactions, stabilization of initiator radicals, and enhancement of endogenous antioxidants (Sachdeva et al., 2014).

In addition, several studies show the health benefits of bioactive components of purple okra, namely increasing antioxidant status, improving oxidative stress conditions, hypoglycemia effects

(Anjani et al., 2018; Nabila et al., 2018), improving lipid profiles (Nabila et al., 2018), improving kidney functions (Wahyuningsih et al., 2021a), improving liver functions (Wahyuningsih et al., 2021b), anticancer effects (Achmad, 2022), and reducing inflammation (Pramudya et al., 2022).

Total Flavonoids

The bioactive component of flavonoids in purple okra extract acts as an antioxidant to repair damage caused by radical compounds (Nabila et al., 2018). The results of the total flavonoids analysis showed a value of 81.32 mg QE g⁻¹ (Table 3). In contrast to research on green okra extract by Chandra et al. (2022) with total flavonoids of 3.19 mg QE g⁻¹. Apart from being caused by differences in okra varieties, the previous study required maceration for 5 days, while this study required 24 hours only. Maceration time of 24 hours has higher bioactive components compared to 12, 36, 48, 60, and 72 hours (Widodo et al., 2021). The longer the maceration time tends to reduce the total flavonoids due to oxidation which damages the flavonoid compounds after the optimum time has passed. The total flavonoids were also different compared to the research by Syam et al. (2020) in extracts from green okra and red okra of 2.57 mg QE g⁻¹ and 2.84 mg QE g⁻¹. Apart from being caused by differences in okra varieties, previous study used a different solvent concentration, namely 70%, while this research used 96%. Solvent concentration can result in changes in solvent polarity, thereby affecting the solubility of bioactive compounds (Suhendra et al., 2019).

Encapsulated purple okra extract has the potential as a nutraceutical with flavonoid content which plays a role not only in improving health status but helps prevent and reduce the consequences of degenerative diseases (Balentine et al., 2015). Flavonoid prevent damage by activating the main antioxidant defense enzymes, activating metal chelators, and inhibiting the activity of enzymes that produce free radicals (Banjarnahor & Artanti, 2014; Panche et al., 2016). According to Procházková et al. (2011), flavonoids easily donate hydrogen atoms to radical compounds resulting in the reduction of highly oxidized radicals. Flavonoid phenoxyl radical can change into aroxyl radical which is capable

of carrying out secondary radical scavenging activities by transferring spare electrons and obtaining a stable structure.

Quercetin

Quercetin is one of the main flavonols from the class of flavonoids which has a hydroxyl group on the benzo-dihydropyran ring which makes quercetin have strong antioxidant activity (Yang et al., 2020). Quercetin can express a higher antioxidant capacity than other flavonoid derivatives (Banjarnahor & Artanti, 2014). The results of the analysis of suspected quercetin derivatives in the encapsulated purple okra extract showed a value of 4.91 mg g⁻¹ (Table 3). The results of the chromatogram using HPLC are shown in Figure 2. The retention time that appeared with the largest area on standard quercetin was 1.213 minutes, while for purple okra extract was 1.111 minutes. Spike results using a quercetin standard on purple okra extract (1:4) showed a new peak that was visible at the retention time of the quercetin standard. Based on the results of identifying quercetin derivatives, it can be assumed that the compound seen in purple okra extract is a quercetin derivative, namely quercetin-3'-O-sulphate (Yang et al., 2018) or quercetin rhamnoside-(feruloyl-hexoside) (Acquavia et al., 2021) with a retention time adjacent to the retention time of quercetin.

The presence of quercetin in okra is qualitatively known but has not been analyzed quantitatively. Green okra is known to contain quercetin of 0.018 mg g⁻¹ (Utami, 2018). The quercetin content was different compared to the green okra extract form in the same study, which was 2.47 mg g⁻¹. The extract form can attract bioactive components so that it has a higher quercetin content. When compared to green okra, purple okra has a different quercetin content of 0.039 mg g⁻¹ in fresh form (Utami, 2018) and 0.45 mg g⁻¹ in extract form (Anjani, 2018). Biofortification of purple okra produces varieties with superior bioactive components (Anjani, 2018).

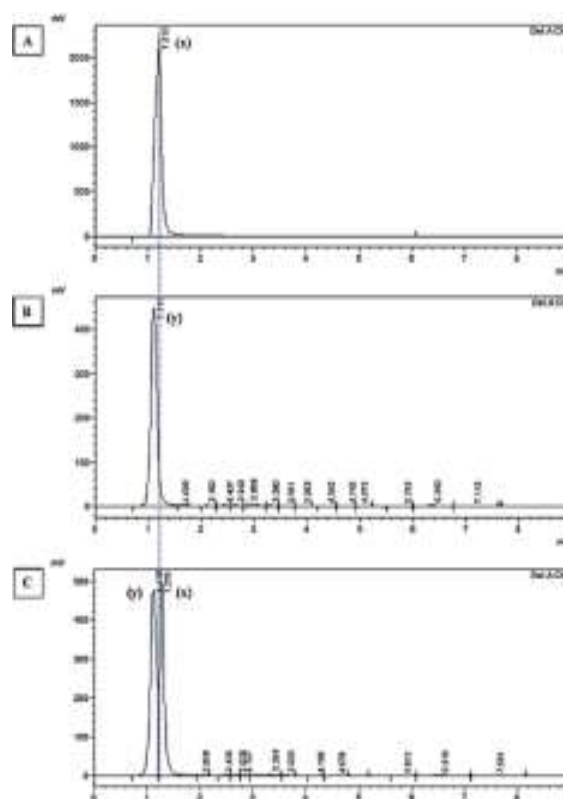


Figure 2. Quercetin Analysis Chromatogram. (A) Quercetin Standard, (B) Purple Okra Extract, (C) Spike of Quercetin Standard and Purple Okra Extract, (x) Quercetin Standard Peak, (y) Suspected Quercetin Derivative Peak

Encapsulated purple okra extract has potential as a nutraceutical with quercetin content which can improve oxidative stress conditions. According to Xu et al. (2019) and Yang et al. (2020), quercetin has effects on signal transduction pathways such as activating, inhibiting, increasing, and downregulating conditions related to oxidative stress. The antioxidant activity of quercetin has been identified primarily through its effect on regulating glutathione peroxidase levels and increasing the expression levels of endogenous antioxidant enzymes. Quercetin also works with its ability to scavenge free radicals including superoxide anions, hydroxyl radicals, and peroxy radicals as well as better inhibition of ROS formation compared to other classes of flavonoids (Demirci, 2017; Elkhalfi et al., 2021).

CONCLUSION

Encapsulated purple okra extract shows its potential as a nutraceutical which has a dry powder form, greenish-brown color, odor characteristic of okra extract (caramel-like), and sour taste with yield of 4%, pH of 4.8, undetectable solvent residue, water content of 13.5%, total ash content of 10.4%, and disintegration time of 1.25 minutes. Microbial contamination showed TPC of 3.1×10^2 CFU g⁻¹, YMC, *E. coli*, and *S. aureus* negative CFU g⁻¹, and *Salmonella spp* negative CFU 10g⁻¹. Heavy metal contamination showed As, Pb, Cd, and Hg contamination not detected per mg Kg⁻¹. Bioactive components such as antioxidant capacity, total flavonoids, and suspected quercetin derivatives showed values of 84.88%, 81.32 mg QE g⁻¹, and 4.91 mg g⁻¹. These bioactive components act as free radical scavengers in helping prevent and reduce the consequences of degenerative diseases. Further research can prove in vivo the potential of encapsulated purple okra extract as a nutraceutical.

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PHYSICOCHEMICAL CHARACTERISTICS, ANTIOXIDANT ACTIVITY AND SENSORY OF COOKIES BASED ON MOCAF, PURPLE YAM, AND CINNAMON FLOUR

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ABSTRACT

In general, cookies are made by using wheat flour with high gluten content, consequently it may cause negative impact on health. So far, cookies also have not the characteristics as functional food that is beneficial for health. Based on these issues, wheat flour is needed to substitute with alternative materials in order to reduce the gluten and increase the functional characteristic of cookies. Mocaf, purple yam flour and cinnamon powder can be used to provide cookies with low gluten and have the characteristic of functional food. The aims of this research are to characterize the physicochemical and organoleptic properties of cookies based Mocaf flour, purple yam flour and cinnamon powder. This work was used he completely randomized design (CRD) with the formulations applied F₀(100:0:0), F₁(75:24.5:0.5), F₂(75:24:1), F₃(75:23.5:1.5), F₄(50:49.5:0.5), F₅(50:49:1), F₆(50:48.5:1.5), F₇(25:74.5:0.5), F₈(25:74:1), and F₉(25:73.5:1.5) with 3 replications. Analysis of physicochemical properties of cookies consists of moisture, fat, total protein, ash, carbohydrates contents, texture, and antioxidant activity. The organoleptic properties were tested by 30 semi-trained panelists. The collected data were analyzed by one way ANOVA at significance level of 5% and followed by Duncan's test. These results show the contents of fat, protein, ash, carbohydrate, water, antioxidant activities and hardness were obtained in the range of 28.1-29.4%, 3.3-3.6%, 1.5-2.2%, 59.8-62.1%, 44.5-88.8 ppm and 13.8 - 38.3 N, respectively. Based on the organoleptic tests, F₂ cookies was most preference than other formulations. The cookies produced believes have low gluten and food functional properties

Keywords: cinnamon, cookies, mocaf, purple yam flour

INTRODUCTION

Public health is the most important factor in supporting the progress of a nation. Good public health conditions play an important role in increasing productivity, education, development, social stability, and security as well as the development of human resources. In Indonesia, the management of health problems is regulated in the Regulation of the Minister of Health of the Republic of Indonesia Number 21 of 2020 concerning health management strategy plans (Putranto, 2020). Health management is comprehensive and reaches all levels of society so that health conditions are more under control.

Currently, several health issues such as celiac and degenerative diseases. Celiac disease is an autoimmune process in the body that is triggered by the habit of consuming foods high in gluten. The body gives an autoimmune reaction to

gluten so that it interferes with the intestines and absorption of nutrients, causes gastrointestinal symptoms, and others. If this disease is not treated properly it can result in complications of various other types of diseases (Oktadiana, Abdullah, & Renaldi, 2017). Meanwhile, degenerative diseases such as heart disease, diabetes mellitus are also very important to overcome.

A good method for overcoming and preventing these diseases is to adopt a healthy diet and get used to consuming functional foods. Functional food is food that has basic nutrition and has a positive effect on health. The ingredients used in the manufacture of functional food must contain basic nutrients and bioactive components that are beneficial to health. Functional food can be prepared using a variety of basic ingredients such as vegetables, fruits, grains, tubers, herbs, and others (Suter, 2013).

One of the food products that can be innovated into functional food products is cookies. Cookies are crispy dry bread with a sweet taste and are usually made from main ingredients such as wheat flour or wheat flour (Herawati, Suhartatik, & Widanti, 2018). Currently, cookies are growing and are available in various variants such as chocolate, peanut, coconut, vanilla, red velvet, blueberry, and others. However, the main ingredients used for making cookies are the same, namely wheat flour or wheat. As it is known that wheat flour (especially high in gluten) is not good for health. Gluten is a protein component composed of gliadin (20-25%) and glutenin (35-40%) (Fitasari, 2009) which are mostly found in cereal foods such as wheat flour (F Kusnandar, Harya, & Agus, 2022)

Apart from the negative impact on health, another disadvantage of high-gluten wheat flour is that it produces dough for cookies that is tough and tough (Masrikhiyah, 2021). Also, the resulting cookie products are not suitable for consumption by people with celiac disease. Patients with celiac disease such as anemia, osteoporosis, dermatitis herpetiformis, neurological symptoms, and diabetes mellitus (Gujral, Freeman, & Thomson, 2012) will detect gluten as a dangerous component, due to changes in the small intestine that result in impaired absorption of nutrients into the body (Permatasari, Ina, & Yusa, 2018). Therefore, people with celiac disease are advised to reduce the consumption of foods that contain gluten (Gujral et al., 2012).

The concept of gluten-free cookies with the theme of functional food as a new food product innovation is very interesting to develop. Indeed, the nature of functional food is not solely determined by high, low, or no gluten content, however, foods that are high in gluten content generally tend to cause adverse effects on health, so they are not suitable to be called functional foods. The concept of gluten-free cookies can be realized by substituting wheat flour with alternative flour (which does not contain gluten) such as modified cassava flour (Mocaf) (Tanjung & Kusnadi, 2015). In addition, the concept of cookies as functional food can also be realized through the use of raw materials that contain other compounds that can provide benefits to the body, such as antioxidant compounds.

It is well known that apart from being high in gluten, cookies usually do not contain (low) antioxidants. Therefore, innovation is needed to overcome this problem. Foodstuffs that can be used as sources of antioxidants include purple yam and cinnamon because these two types of ingredients are rich in antioxidants. According to research results Prasetyo and Winardi (2020) that the antioxidant activity (based on radical scavenging activity, RSA) in fresh purple yam was around 62.14%, and purple yam flour was around 20.19%. Whereas in cinnamon bark the antioxidant activity (based on the inhibition concentration, IC₅₀) is around 1.94 ppm (Antasionasti & Jayanto, 2021).

Gluten-free and high-antioxidant cookies have several advantages, including being suitable for consumption by people with celiac disease and being beneficial for the body as an antidote to free radicals. Many studies have been reported on the use of Mocaf flour, purple yam flour, and cinnamon powder for making cookies, such as research on making cookies. For example, (Herawati et al., 2018; Rasyid, Maryati, Triandita, Yuliani, & Angraeni, 2020) were used Mocaf flour, Fitriani, Yurnalis, and Hermalena (2019) used purple yam flour and white yam(40:60) and Fairus, Hamidah, and Setyaningrum (2021) were used Mocaf flour, purple yam and peanuts (20:35:45). The physicochemical characteristics of the cookies produced such as moisture, fat, protein, ash, and carbohydrate contents were obtained in the range of 1.1 - 3.1%, 12.7 - 25.2%, 2.9 - 12.6%, 0.9 - 1.1%, and 29.6%, respectively. Meanwhile, the results of research on antioxidant activity in cookies made from purple yam flour and peanuts are reported by Martins, Susilowati, and Jinarti (2014) where the percentage of radical scavenging activity (%RSA) is around 39.7%.

The use of local food commodities can reduce the need for wheat flour (Tamaroh & Sudrajat, 2021). Many local food commodities have not been used optimally, such as cassava (*Manihot esculenta*) and purple yam (*Ipomoea batatas*). Cassava can be processed through fermentation to produce gluten-free flour known as Mocaf. Meanwhile, purple yam (*Ipomoea batatas L.*) can be processed into antioxidant-rich flour (Nabilah, 2019). The use of cinnamon (*Cinnamomum*

burmannii) as an additional ingredient in making cookies is also an interesting innovation because cinnamon is rich in antioxidant compounds such as eugenol, safrole, cinnamaldehyde, tannins (Hariana, 2007), and polyphenol (Priani, Darusman, & Humanisya, 2014).

Parameters of cookie quality involving physicochemical and sensory properties are very important to evaluate before the cookies are commercialized. Quality cookies are cookies that meet physicochemical and sensory quality criteria. In general, the physicochemical properties that are often evaluated include moisture content, protein, fat, ash, carbohydrates and texture. Based on SNI-2973-2018 that the maximum water content is 5%, the minimum protein is 9%, the minimum fat is 9.5%, the maximum ash is 1.5% and carbohydrates is maximum 70% (BSN, 2018). In addition, according to Rahardjo, Nugroho, and Saibele (2021) the sensory also determines the quality of cookies. Sensory parameters evaluated include aroma, taste, color, and texture.

Based on the illustration above, the scenarios are needed to produce gluten-free cookie products that have functional food characteristics. This research tries to the use of Mocaf and purple yam flour as basic ingredients for making cookies, and cinnamon powder as an additional ingredient. The purpose of this study is to determine the physicochemical characteristics, antioxidant activity, and sensory activity of cookies made from Mocaf flour and purple yam flour with added cinnamon powder. In addition, this study also aims to provide information related to the formulation of good cookies based on the nutritional, antioxidant, and sensory aspects. Several physicochemical properties of cookies were analyzed such as moisture content, fat, total protein, ash, carbohydrates, hardness, and antioxidant activity. Meanwhile, the sensory properties of the cookies that were evaluated included color, taste, aroma, texture, and overall preferences.

MATERIALS AND METHODS

Materials and Instruments

The materials were cassava, purple yam and cinnamon. Other materials needed were margarine, sugar, eggs, skimmed milk, and baking powder.

All materials were purchased from the Yogyakarta Traditional Market. The instruments used were a sieve, blender, rolling pin, Soxhlet, UV-Vis Spectrophotometer (Thermoscientific), Oven (Memmert), and UTM (Zwick/z0.5).

Mocaf flour Preparation

The preparation of Mocaf flour was performed by using a method as described by Yani and Akbar (2018). A total of 1 kg of cassava peeled was washed with water and sliced using grated chips. After that, the cassava slices were soaked in water, added a total of 5 g of yeast (*Saccharomyces cerevisiae*), and left for 12 hours. Then, the cassava slices were drained and dried in an oven at 60°C for 24 hours. Dried cassava slices were fined by using a blender for 15 minutes and then sieved through an 80 mesh. The Mocaf flour product was shown in **Figure 1**.



Figure 1. Mocaf flour.

Purple Yam Flour Preparation

The preparation of purple yam flour was performed using a method as described by Anggarawati, Ekawati, and Wiadnyani (2019). A total of 1 kg of sorted purple yam was peeled, washed with clean water, sliced with grated chips, placed on a baking sheet, and dried in an oven at 60°C for 4 hours. Then, the dried slices were fined with a blender and sieved through a 60 mesh. The product of purple yam flour was shown in **Figure 2**.



Figure 2. Purple yam flour

Cinnamon Powder Preparation

The preparation of cinnamon powder was performed as described by Shahid et al. (2018). A total of 75 g of cinnamon bark was washed with clean water, cut into small pieces, and dried in an oven at 60°C for 6 hours. After that, the dried cinnamon pieces were mashed using a blender and sieved through a 60 mesh. The product of cinnamon powder was shown in **Figure 3**.



Figure 3. Cinnamon powder

Experimental Design

This work was designed by a completely randomized design (CRD) with a comparison composition of Mocaf flour (M), purple yam (PY), and cinnamon powder (C). In detail, **Table 1** was presented the research experimental design in this work.

Table 1. Experimental design in this work

Formulation (%)			Symbols	Repetitions		
M	PY	C		1	2	3
100	0	0	F ₀	F ₀₁	F ₀₂	F ₀₃
75	24,5	0,5	F ₁	F ₁₁	F ₁₂	F ₁₃
75	24	1	F ₂	F ₂₁	F ₂₂	F ₂₃
75	23,5	1,5	F ₃	F ₃₁	F ₃₂	F ₃₃
50	49,5	0,5	F ₄	F ₄₁	F ₄₂	F ₄₃
50	49	1	F ₅	F ₅₁	F ₅₂	F ₅₃
50	48,5	1,5	F ₆	F ₆₁	F ₆₂	F ₆₃
25	74,5	0,5	F ₇	F ₇₁	F ₇₂	F ₇₃
25	74	1	F ₈	F ₈₁	F ₈₂	F ₈₃
25	73,5	1,5	F ₉	F ₉₁	F ₉₂	F ₉₃

Cookies Preparation

The composition of the ingredients for making cookies were presented in **Table 2**. Margarine, fine sugar, and egg yolks were mixed by using a mixer for 3 minutes. Then, the mixtures were homogenized by using a mixer for 2 minutes. After that, the mixtures were added with the baking powder, skim milk, Mocaf flour, purple, and cinnamon powder. Again, the mixture was then homogenized with a mixer for 5 minutes. The dough was printed on the brass and baked in the oven at 180°C for 13 minutes (Waisnawi, Yusasrini, & Ina, 2019).

Table 2. Cookies ingredient

Ingredients (g)	Formulations									
	F ₀	F ₁	F ₂	F ₃	F ₄	F ₅	F ₆	F ₇	F ₈	F ₉
Margarine	85	85	85	85	85	85	85	85	85	85
Fine Sugar	60	60	60	60	60	60	60	60	60	60
Egg yolk	10	10	10	10	10	10	10	10	10	10
Baking powder	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5
Skimmed milk	15	15	15	15	15	15	15	15	15	15
Mocaf flour	100	75	75	75	50	50	50	25	25	25
Purple yam flour	0	24,5	24	23,5	49,5	49	48,5	74,5	74	73,5
Cinnamon powder	0	0,5	1	1,5	0,5	1	1,5	0,5	1	1,5

Hardness Test

The texture was tested based on the level of hardness. The hardness level was tested by using the Universal Testing Machine (UTM, Zwick/z0.5). The levels of hardness were measured based on the amount of force needed to break or penetrate the cookies (Bourne, 2002).

Water content measurement

The water content of cookies was determined by using the AOAC (2005) method. The crucible was primarily dried in the oven at 105°C for 1 hour. The cup was then cooled in a desiccator for 15 minutes and then weighed until the weight was constant. A total of 2 g of sample was placed in a cup and dried in the oven at 105°C for 24

hours. After that, the samples were cooled in a desiccator for 15 minutes and then weighed. The water content was determined using the following Equation 1;

$$C_W = \frac{W_1 - W_2}{W_1} \times 100\% \dots (1)$$

Where;

C_W = water content (%); W_1 = weight of the cup and sample before dried (g); W_2 = weight of the cup and sample after dried (g)

Fat content measurement

Fat content was determined by using the Soxhlet method as described in the AOAC (2005). The distillation flask (filled with boiling stones) was dried in the oven at 105°C, cooled, and weighed. Then, the distillation flask was filled with 10 mL of petroleum benzene solvent. At the same time, the cookie samples were crushed, weighed, put in an extraction sleeve, and covered with cotton. Then, the Soxhlet apparatus was installed and the casing containing the sample was placed in the sample holder. The extraction process was carried out at 60-70°C for 6 hours. After the extraction process was completed, the solvent was removed using a rotary evaporator. Lastly, fat content was determined using the following Equation 2;

$$C_F = \frac{W_o - W_d}{W_s} \times 100\% \dots (2)$$

Where;

C_F = fat content (%); W_d = weight of flasks and boiling stone (g); W_o = weight of fat, flask, and boiling stone (g); W_s = weight of the sample (g)

Measurement of Total Protein Content

Total protein content was determined using the Kjeldahl method as described in the AOAC (2005). Three stages were carried out including the stages of destruction, distillation, and titration. A total of 0.25 g of the cookie sample was crushed, and put into a 100 mL Kjeldahl flask, added 0.7 g of catalyst N (K_2SO_4 : $CuSO_4$, 2:3) and 4 mL of concentrated H_2SO_4 (98%). The sample destruction process was carried out by heating at 410°C for 1 hour in a fume hood until the color of the solution

was changed to clear green. Then, the solution was cooled, added 50 distilled water and 20 mL of 40% NaOH.

The distillation process was carried out at 100°C. The distillate was collected in an Erlenmeyer containing 10 mL of boric acid solution (H_3BO_3 , 2%) and 3 drops of (bromocresol green methyl red, BCG-MR) indicator. Once the color of the distillate was changed from red to blue and the volume reached 40 ml, the distillation process was stopped. Then, the distillate was titrated with 0.01 N HCl until the pink color appeared. Also, the titration process on blank was performed. Record the volume of titrant used to titrate the sample and the blank. Protein levels were determined using the following Equation 3;

$$C_p = \frac{(V_2 - V_1) \times N \times 0.014 \times F_k \times F_p}{W} \times 100\% \dots (3)$$

Where;

C_p = protein content (%); V_1 = volume of titrant for blank (mL); V_2 = volume of titrant for sample (mL); N = normality of HCl (0.01 N); F_p = dilution factor, F_k = conversion factor (6,25)

Ash Content Measurement

The ash content was determined by using the procedure of AOAC (2005). The crucible was dried in the oven at 105°C for 1 hour., cooled in a desiccator for 15 minutes, and then weighed until the weight was constant. A total of 2 g of sample was put into a crucible and the burning process was carried out in a furnace at 600°C for 3 hours. Then, the burning process was stopped, and allowed to cool down to 120°C. The crucible was removed from the furnace and cooled in a desiccator for 15 minutes. Finally, the crucible and ashes were weighed. The ash content was calculated using the following Equation 4;

$$C_{Ash} = \frac{W_1 - W_2}{W_1} \times 100\% \dots (4)$$

Where;

C_{Ash} = ash content (%); W_1 = weight of crucible and sample (g); W_2 = weight of crucible and ash (g).

Carbohydrate Content Calculation

Analysis of carbohydrate content can be determined based on the difference of 100% of total content minus the water, fat, protein, and ash contents. Equation 5 can be used to calculate carbohydrate content.

$$C_C = 100\% - (W + F + P + Ash)\% \dots (5)$$

Where;

C_C = carbohydrate content (%); W = water content (%); F = fat content (%); P = protein content (%); Ash = ash content (%)

Antioxidant Activity (IC₅₀) Analysis

The procedure for analyzing the antioxidant activity was carried out by using the method as described by Indriyani, Nurhidajah, and Suyanto (2013). The solution of 0.2 M DPPH was prepared by dissolving 0.8 g of DPPH powder (BM 394.32 g/mol) in 10 mL of methanol. Then, a total of 0.1 ml of the 0.2 M DPPH solution was taken and put in a 100 ml volumetric flask, then diluted with methanol to obtain a 0.2 mM DPPH. After that, a total 1 ml of 0.2 mM DPPH solution was taken, put in a test tube, and add 4 ml of methanol, homogenized using a vortex and incubated for 30 minutes. Finally, determine the absorbance of the solution (blank) using a UV-Vis spectrophotometer (Thermo Scientific) at a wavelength of 517 nm.

The sample preparation was carried out by dissolving 10 mg of cookies in 10 mL of methanol in a test tube. Then, the sample solution was prepared in the series concentration of 100 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm, respectively. A total of 1 mL of each sample solution was taken, add 1 mL of 0.2 mM DPPH solution, placed in different test tubes, and diluted with methanol up to 5 mL of total volume. Then, the mixture was homogenized using a vortex and incubated for 30 minutes. Lastly, sample absorption was measured using a UV-Vis spectrophotometer (Thermo Scientific) at a wavelength of 517 nm. The percentage of inhibition was calculated by using Equation 6.

$$\text{Inhibition (\%)} = \frac{A_b - A_s}{A_b} \times 100\% \dots (6)$$

Where;

A_b = absorption of the blank; A_s = absorption of the sample

Antioxidant activity (IC₅₀) was determined by using the calibration curve from the inhibition percentage. The percentage of inhibition was plotted as the y-axis and the \ln of the concentration was plotted as the x-axis so that Equation 6 was obtained. Then, the IC₅₀ was determined using Equation 7.

$$y = ax + b \dots (6)$$

$$50 = ax + b$$

$$x = \frac{50 - b}{a}$$

$$IC_{50} = \text{anti} \ln x \dots (7)$$

Where;

a = slope; b = intercept; x = concentration of antioxidant (ppm)

Sensory Test

The sensory properties of cookies such as color, taste, aroma, texture, and overall level of preference were evaluated by involving 30 untrained panelists. The number of untrained panelists can be selected around 25 - 50 people to get good sensory test results (Meilgaard, Gail Vance Civile, & Carr, 2007). The panelists in this study were selected from healthy students consisting of 15 boys and 15 girls with an average age of 20-21 years. Scoring based on preference levels were 1 (dislike very much), 2 (dislike), 3 (neutral), 4 (like), and 5 (very like).

Data Analysis

The data obtained were analyzed by using SPSS software version 25.0. with a one-way ANOVA test at a significance level of 5% ($\alpha = 0.05$). If there was a significant difference between the treatments, then a further test was carried out using Duncan's test.

RESULTS AND DISCUSSION

Cookies and Their Physicochemical Properties

Overall, the physicochemical characteristics of cookies based on Mocaf flour, purple yam flour, and cinnamon were shown in **Table 3**. As a comparison, some relevant research literature was also presented.

The types of cookie products produced were shown in **Figure 4**. Visually, the cookies showed that the color of the cookies gets darker as

the purple yam flour composition increases. This can be caused by the purple pigment of the purple sweet potato.

Table 3. Physicochemical properties of cookies

Sample	Water content (%)	Fat content (%)	Total Protein (%)	Ash content Abu (%)	Carbohydrate content (%)	Hardness (N)
F0	4,8 ± 0,1 ^a	28,5 ± 0,2 ^{ab}	3,4 ± 0,1 ^b	1,5 ± 0,2 ^a	61,8 ± 0,2 ^{de}	35,3 ± 3,5 ^{de}
F1	4,9 ± 0,1 ^{ab}	28,2 ± 0,2 ^a	3,3 ± 0,0 ^a	1,6 ± 0,1 ^a	62,1 ± 0,1 ^c	21,6 ± 4,2 ^{bc}
F2	4,9 ± 0,1 ^{ab}	28,5 ± 0,3 ^{ab}	3,3 ± 0,1 ^a	1,6 ± 0,1 ^{ab}	61,8 ± 0,5 ^{de}	18,8 ± 3,2 ^{ab}
F3	4,9 ± 0,2 ^{ab}	28,6 ± 0,2 ^{ab}	3,3 ± 0,0 ^a	1,6 ± 0,0 ^{ab}	61,6 ± 0,4 ^{cde}	13,8 ± 2,3 ^a
F4	4,9 ± 0,2 ^{ab}	28,4 ± 0,1 ^{ab}	3,5 ± 0,0 ^{bc}	1,6 ± 0,0 ^{ab}	61,5 ± 0,1 ^{cde}	30,3 ± 0,5 ^d
F5	4,9 ± 0,1 ^{ab}	28,8 ± 0,3 ^{abc}	3,5 ± 0,0 ^{bc}	1,8 ± 0,2 ^b	61,0 ± 0,4 ^{bc}	24,9 ± 2,0 ^c
F6	4,9 ± 0,0 ^{ab}	29,1 ± 0,9 ^{bc}	3,5 ± 0,1 ^{bc}	1,9 ± 0,0 ^c	60,6 ± 0,9 ^b	17,9 ± 2,3 ^{ab}
F7	5,0 ± 0,1 ^b	28,1 ± 0,2 ^a	3,5 ± 0,1 ^{bc}	2,1 ± 0,0 ^{cd}	61,2 ± 0,2 ^{bcd}	38,3 ± 0,5 ^c
F8	5,0 ± 0,2 ^{ab}	28,7 ± 0,3 ^{ab}	3,5 ± 0,0 ^{bc}	2,2 ± 0,0 ^{cd}	60,6 ± 0,1 ^b	35,1 ± 0,7 ^{de}
F9	4,9 ± 0,1 ^{ab}	29,4 ± 0,3 ^c	3,6 ± 0,0 ^c	2,2 ± 0,0 ^d	59,8 ± 0,3 ^a	35,0 ± 5,9 ^{de}
Rata-rata	4,9 ± 0,1	28,6 ± 0,5	3,4 ± 0,1	1,8 ± 0,2	61,2 ± 0,8	27,1 ± 8,8
Referensi	Max. 5%*	Min. 9,5%*	Min. 5%*	Max. 1,6%*	Min. 70%*	22-50 N**

Note: Numbers followed by the same superscript letter indicate no significant difference;* Source (Nasional, 2011); ** Source (Nindiyarani, Sutardi, & Suparmo, 2011).

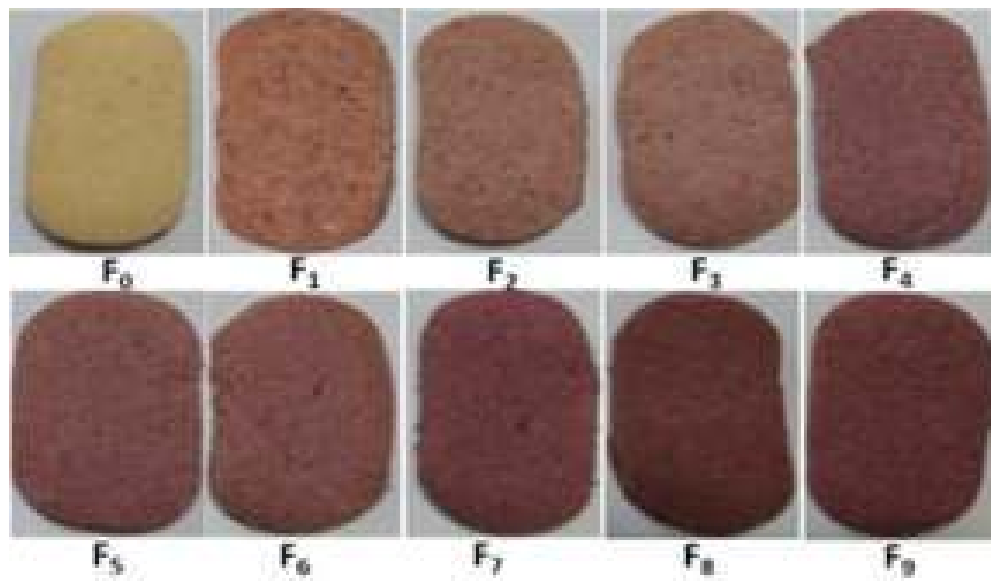


Figure 4. Cookies based on Mocaf flour, purple yam flour and cinnamon powder

Water content

Water content indicates the proportion of water composition in a food ingredient. Based on **Table 3**, the F₇ shows the highest level of water content which was obtained at around 5.0%, while the F₀ shows the lowest level of water content which was around 4.8%. The high

or low water content is caused by the ability of raw materials to absorb the water. Mocaf and purple yam flour have a greater water absorption capacity than wheat flour (Etudaiye, Oti, Aniedu, & Omodamiro, 2015). The water absorption capacity of Mocaf flour and purple yam were around 250 – 300% (Olatunde, Henshaw, Idowu,

& Tomlins, 2016), while wheat flour is around 50 – 60% (Feri Kusnandar, Danniswara, & Sutriyono, 2022). One of the important factors that affect the absorption of water in food is the protein content. The side chain polar groups of protein compounds such as carbonyl, hydroxyl, amino, carboxyl, and sulfhydryl are hydrophilic components, so they can hydrogen bond with water (Rauf & Sarbini, 2015). As shown in **Figure 5**, the water content tends to increase with increasing protein content in cookies. However, in general, the water content in each formulation was not significantly different ($p>0.05$).

Reference to the quality requirements for cookies based on SNI 01-2973-2011 where the maximum water content is around 5% (Badan Standarisasi Nasional, 2011). The water content for all formulations meet the specified criteria where the moisture content of cookies was in the range of 4.8-5.0%.

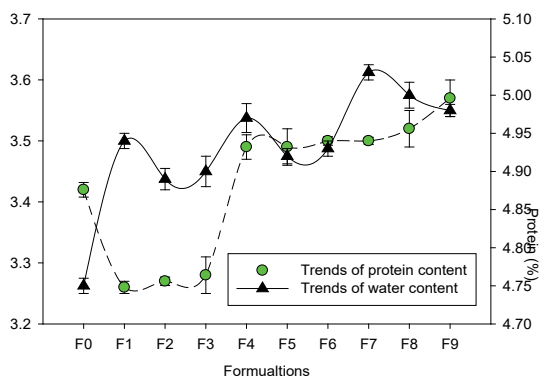


Figure 5. Correlation between protein and water contents

Fat content

Based on **Table 3**, the fat contents were obtained in the range of 28.1 - 29.4%. These fat contents were much higher than the standard set by SNI 01-2973-2011 where the minimum cookie fat content is around 9.5% (Nasional, 2011). These facts show the resulting cookies meet the standards set.

Overall, the fat content in each formulation was not significantly different ($p>0.05$). This fact might be due to the fat content in Mocaf flour and purple yam flour being quite the same, which was in the range of 0.4 – 0.8 g per 100 g, consequently, the changes in composition do

not have a significant effect on the fat content of cookies. In addition to the fat content in Mocaf and purple yam flour, the cinnamon composition also affects the fat content of cookies. According to Singh, Maurya, Delampasona, and Can (2007) that cinnamon contains essential oils which can be categorized as fats (Mulyani & Sujarwanta, 2018). In general, although not significant, the increase in the composition of purple yam flour and cinnamon powder tends to increase the fat content of cookies. The phenomenon of the effect of adding purple yam flour and cinnamon powder on the increase in fat content was shown in **Figure 6**. The higher composition of purple yam flour and cinnamon powder tends to increase the fat content in the cookies.

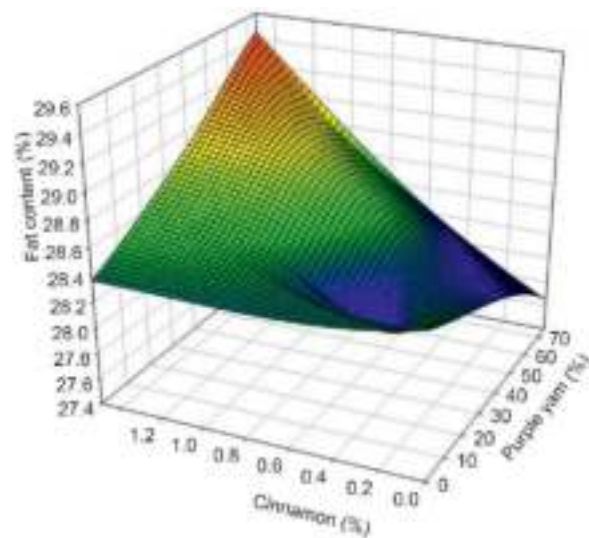


Figure 6. Correlation between purple yam flour and cinnamon powder compositions on fat content in cookies

Protein Total Content

Protein is one of macronutrients that is the most important for the body. Protein content can help repair muscles and create a feeling of fullness. However, consuming cookies that are rich in protein must be limited to prevent a negative impact on health. Based on **Table 3**, the protein contents were obtained in the range of 3.3 - 3.6%. These protein contents were lower than the standard set in SNI 01-2973-2011 which is 5%. The low protein content may be caused by the low protein content in Mocaf, purple yam flour, and cinnamon powder. The highest protein content was found in F₉ while the lowest in F₁. Based

on **Figure 7**, protein content tends to increase with increasing composition of purple yam flour and cinnamon. An increase in the composition of purple yam flour and cinnamon powder had a significant effect on protein content ($p < 0.05$). This means the higher the composition of purple yam flour and cinnamon powder, so the higher the protein content of cookies. This might be due to the protein content in purple yam flour (3.8%) and cinnamon (2.5%). As described above, the F_9 formulation uses the most purple yam flour and cinnamon powder, so the protein content was the highest (3.6%).

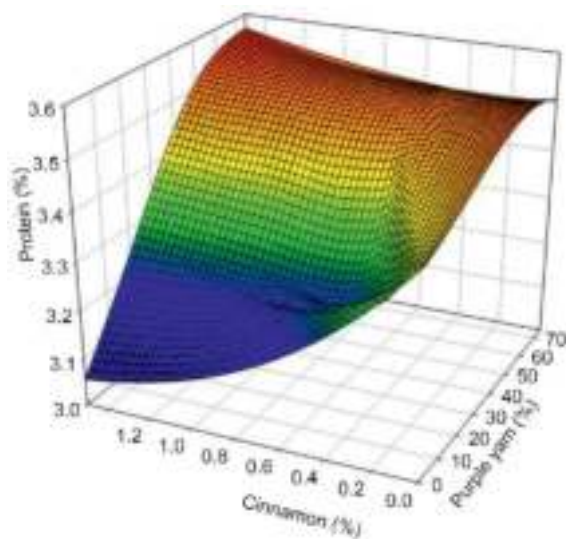


Figure 7. Effect of purple yam flour and cinnamon powder compositions on the changes in protein content

Ash content

Although the ash content does not have a direct effect on health, it needs to analyze to find out a general description of the mineral content in food products. The ash content in food refers to the minerals that remain after all the organic compounds have been burned during the ashing process. Table 3 shows the ash content of cookies, where the highest content was around 2.2% (F_9), while the lowest was around 1.5% (F_0). These results illustrated that the ash content was affected by the high composition of purple yam flour and cinnamon powder. Based on the preliminary test, it was known that the ash content in purple yam was around 1.8%. So that the highest formulation of purple yam flour will have the highest ash content.

In addition, the use of cinnamon powder also had a significant effect ($p < 0.05$) on the ash content. It was well known that cinnamon powder contains the highest ash compared to other ingredients, which was around 4.0%. This fact was caused by cinnamon powder containing calcium oxalate, glycyrrhizin, asparagine, essential oils, and other components (Herawati et al., 2018). As shown above, F_0 has the lowest ash content because Mocaf flour contains the lowest ash content compared to other ingredients, which was 0.4%. The correlation between the composition of purple yam potato flour and cinnamon powder on ash content were shown in **Figure 8**.

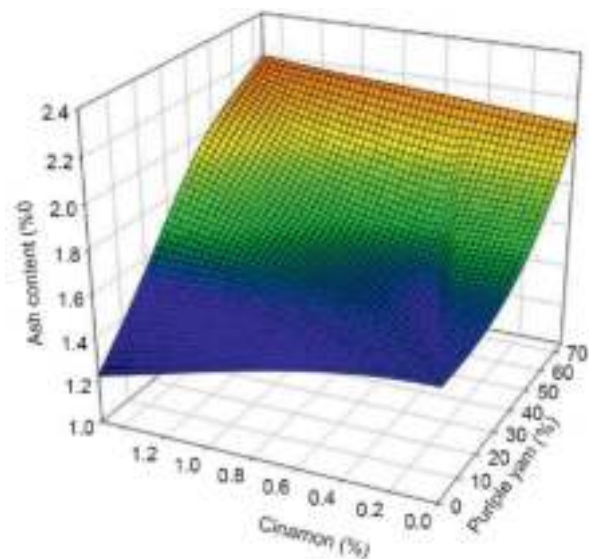


Figure 8. Effect of purple yam flour and cinnamon powder compositions on ash content

The standard of the quality requirements of cookies based on ash content has been stipulated in SNI 01-2973-2011, where the maximum ash content of cookies is 1.6% (Nasional, 2011). The results of the analysis showed that the formulations of F_0 (1.5%), F_1 (1.6%), F_2 (1.6%), and F_3 (1.6%) met the quality requirements for ash content which were obtained around 1.6%. Meanwhile, the formulations of F_4 , F_5 , F_6 , F_7 , F_8 , and F_9 were not meet with the standard ash content ($> 1.6\%$) set.

Carbohydrate content

Based on the quality requirements of cookies according to SNI 01-2973-2011 that the minimum carbohydrate content that must be fulfilled by cookies is around 70% (Nasional, 2011). As

shown in **Table 3** that the carbohydrate contents of cookies were lower than the standard set where the carbohydrate content was in the range of 59.8 - 62.1%. The low carbohydrate content in cookies might be due to the low carbohydrate content in the raw materials of both Mocaf flour and purple yam flour.

In the context of carbohydrate content, Mocaf flour has undergone quality improvements (Kurniati, Aida, Gunawan, & Widjaja, 2012). During the fermentation process, the starch from cassava flour can be hydrolyzed into maltose and then converted into sugar, as a result, Mocaf flour contains higher carbohydrates (Yani & Akbar, 2018). Therefore, the high composition of Mocaf flour tends to increase the carbohydrate content of cookies. The correlation between the composition of Mocaf flour and purple yam on carbohydrate content was shown in **Figure 9**.

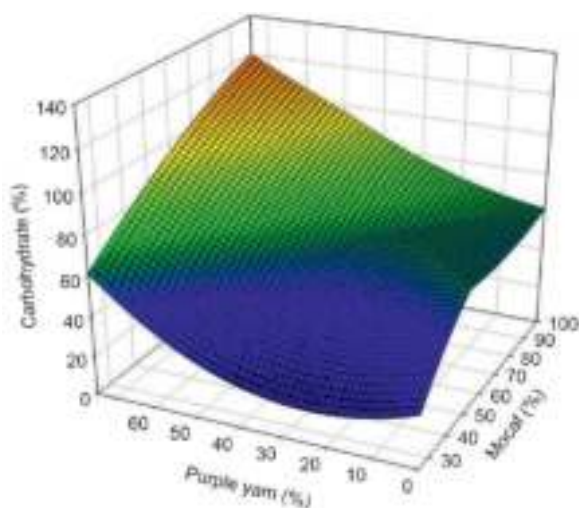


Figure 9. Effect of Mocaf flour and purple yam composition on carbohydrate content in cookies

Hardness

Texture such as hardness is one of the physical parameters that need to be evaluated because it greatly affects the quality of cookies. Cookies that are too hard will be difficult to chew and can reduce the delicious taste. In addition, textures that are too hard also tend to be more brittle and easily crushed, consequently reducing the shelf life. So far, there is no standard hardness value set for cookies, so generally, the results of texture identification are only used to provide information related to the relationship between the formulation

and the hardness value. As shown in Table 3, the highest hardness value was obtained at around 38.3 N (F₇), while the lowest hardness value was obtained at around 13.8 N (F₃). The hardness of cookies was generally affected by water, fat, carbohydrate, and protein contents.

According to Istinganah, Rauf, and Widyaningsih (2017) that starch content (especially amylose) was very associated with the level of hardness. Flour with a high amylose composition tends to produce cookies that were much harder than flour with a low amylose content. Purple yam flour contains about 74.6% starch with 24.8% amylose (Nindyarani et al., 2011) which is higher than the starch content in Mocaf flour which is around 63.1% with an amylose content of around 11.1% (Yani & Akbar, 2018). Therefore, the high composition of purple yam flour tends to increase the hardness level. The phenomenon of the purple yam effect on the level of hardness was shown in **Figure 10**.

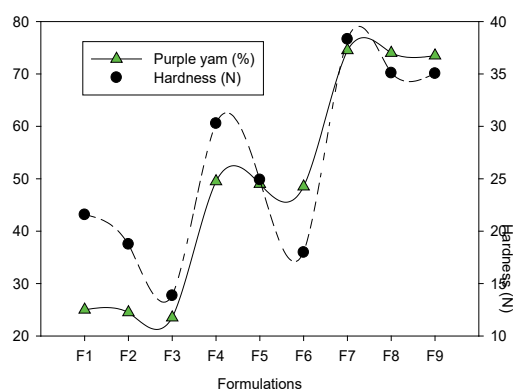


Figure 10. The pattern of increasing the hardness value of cookies was based on increasing the composition of purple yam flour

Antioxidant Activities

The high value of antioxidant activity can describe the functional characteristics of cookies. It is well known that high antioxidant activity has a high ability to counteract free radicals. Antioxidant compounds can prevent the occurrence of an oxidation reaction by preventing the formation of radicals.

Table 4 shows the IC₅₀ values for each formulation. The value of antioxidant activity (IC₅₀) of cookies was obtained in the range of 44.5 - 88.8 ppm. The lowest IC₅₀ value (very strong)

was shown by F₉, while the highest (strong) was shown by F₁. This fact indicates that increasing the composition of purple yam and cinnamon can increase antioxidant activity. This might be due to the purple yam and cinnamon contain various antioxidant compounds. Purple yam contains anthocyanins, β -carotene, vitamin C, and vitamin E which act as antioxidants. Meanwhile, cinnamon contains polyphenols, flavonoids, and caffeine which also act as antioxidant compounds.

The antioxidant activity in purple yam flour was about 83.7% with anthocyanin levels were around 391.1 mg GAE/100 g (Tamaroh & Sudrajat, 2021). Meanwhile, cinnamon contains antioxidant compounds, especially cinnamtannins B1 and B2, oligomeric procyanidins, and proanthocyanidins. The antioxidant activity (%RSA) of cinnamon powder was around 90.0% with an antioxidant level of around 355.0 mg GAE/100 g (Shahid et al., 2018).

According to the research results of Fitriani *et al.* (2019) regarding the antioxidant activity in cookies using 80% purple yam flour (without cinnamon powder) showed the IC₅₀ value was obtained around 75.3 ppm (strong antioxidant activity). Whereas, in this study, the F₇ cookies with a purple yam flour composition of 74.5% show a much lower IC₅₀ value (stronger activity), which was around 50.6 ppm. This fact indicated that the addition of cinnamon powder contributed significantly ($p < 0.05$) to the increase in antioxidant capacity.

Table 4. Antioxidant activities of cookies

Sample	IC ₅₀ (ppm)	Categories
F ₀	88,7 ± 0,6 ^g	Strong
F ₁	88,8 ± 0,5 ^g	Strong
F ₂	80,2 ± 0,3 ^f	Strong
F ₃	74,9 ± 1,3 ^e	Strong
F ₄	67,3 ± 0,8 ^d	Strong
F ₅	66,0 ± 1,5 ^d	Strong
F ₆	55,7 ± 1,7 ^c	Strong
F ₇	50,6 ± 0,2 ^b	Strong
F ₈	49,5 ± 0,8 ^b	Very strong
F ₉	44,5 ± 1,6 ^a	Very strong
Reference	70-8 ppm*	Strong
	62,3 ppm**	Strong

Note: Numbers followed by the same superscript letter indicate no significant difference; * Source (Fitriani et al., 2019); **Source (Hati, Setiani, & Bintoro, 2020)

Sensory Evaluation

The sensory properties of cookies including color, taste, texture, aroma, and overall preference were presented in **Table 5**. Color is a parameter attached to cookies that is first seen (Tarwendah, 2017). Based on the color analysis the color F₇ was the most preferred, where the level of preference was around 3.9 (like criteria). While the color F₀ was the least preferred with a preference level of around 3.1 (neutral category). The appearance of a strong purple color on F₇ makes cookies more attractive to attention than F₀ which was brown (See Figure 1). According to Nabilah *et al.* (2019) which stated that the most preferred color of cookies was cookies with the highest purple sweet potato flour composition, namely 75%.

Taste is one of the sensory parameters of cookies. The taste of the F₉ formulation was the most preferred where the level of preference was observed around 4.1 (like), while the F₀ formulation was least preferred with a level of preference of around 3.4 (neutral). According to Widyasitoresmi (2010) that purple yam has a distinctive taste and tends to be sweet so it was preferred over Mocaf flour which does not have a distinctive or sweet taste (Setyadjid & Setyaningrum, 2022). In addition to purple yam flour, increasing the composition of cinnamon powder can also improve the taste and flavor of cookies because cinnamon powder contains cinnamaldehyde compounds which act as flavor and flavor formers (Shobur, Hersoelistyorini, & Syadi, 2021). Thus, the high composition of purple yam flour and cinnamon powder (formulation F₉) resulted in a balanced taste that the panelists preferred.

As with color and taste, aroma is also an important parameter in food products. Aroma is a parameter attached to food products that can be identified using the sense of smell. Based on the aroma evaluation that the F₉ formulation was the most preferred with a preference level of around 3.8 ± 0.8 (liking criteria). This might due to the aroma of purple sweet potato flour was not so strong. In addition, the use of cinnamon powder in the composition also makes the aroma more attractive. While the F₀ formulation was the least preferred with a preference level of around 3.2 ± 1.0 (neutral criteria). This might be caused by the

emergence of a sour aroma caused by the high composition of Mocaf flour. Mocaf flour has a distinctive cassava flavor and a slightly sour aroma (Yani & Akbar, 2018), as a result, the higher the Mocaf flour composition causes the stronger the sour aroma in the cookies.

Texture parameters also greatly affect the quality of cookies. The harder or softer the texture of the cookies describes the worse the quality. Texture can be judged by biting, chewing, and touching. Based on the texture analysis, the F₂ was the most preferred cookie with a preference level of around 3.9 ± 0.7 (like criteria), while the F₇ was the least preferred with a preference level of around 3.5 ± 0.9 (neutral criteria). Reducing

the composition of purple yam flour (decreasing amylose content) resulted in a decrease in the level of hardness. Formulations F₂ and F₃ used the purple yam flour at 24% and 23.5%, respectively, so their textures were less hard. The level of preference decreased with increasing the purple yam flour composition because the cookies tended to be harder. According to the result that was reported by (Setyadjid & Setiyaningrum, 2022) that cookies with a low composition of purple yam flour (30%) were the most preferred texture. In addition, the results of Nindyarani et al. (2011) also stated that cookies with a low purple sweet potato flour composition (about 25%) were the most preferred.

Table 5. Sensory of cookies

Sample	Hedonic tests				
	Color	Taste	Texture	Aroma	Overall
F ₀	3,1 ± 1,3 ^a	3,4 ± 1,0 ^a	3,6 ± 1,1 ^a	3,2 ± 1,0 ^a	3,4 ± 1,1 ^a
F ₁	3,4 ± 0,8 ^{ab}	3,6 ± 0,9 ^{ab}	3,7 ± 0,6 ^a	3,5 ± 0,7 ^{ab}	3,8 ± 0,8 ^a
F ₂	3,4 ± 0,9 ^{ab}	3,8 ± 0,9 ^{ab}	3,9 ± 0,7 ^a	3,6 ± 0,7 ^b	3,9 ± 0,7 ^a
F ₃	3,2 ± 0,9 ^a	3,8 ± 1,0 ^{ab}	3,9 ± 0,7 ^a	3,6 ± 0,6 ^{ab}	3,7 ± 1,0 ^a
F ₄	3,8 ± 0,6 ^b	3,9 ± 0,8 ^{ab}	3,7 ± 0,9 ^a	3,7 ± 0,7 ^b	3,8 ± 0,7 ^a
F ₅	3,8 ± 0,7 ^b	3,8 ± 0,9 ^{ab}	3,7 ± 0,9 ^a	3,6 ± 0,7 ^{ab}	3,7 ± 0,8 ^a
F ₆	3,8 ± 0,9 ^b	3,9 ± 0,9 ^{ab}	3,7 ± 0,7 ^a	3,7 ± 0,5 ^b	3,9 ± 0,8 ^a
F ₇	3,9 ± 0,9 ^b	4,0 ± 0,9 ^b	3,5 ± 0,9 ^a	3,6 ± 0,9 ^{ab}	3,6 ± 1,0 ^a
F ₈	3,8 ± 0,9 ^b	3,9 ± 0,9 ^{ab}	3,6 ± 0,9 ^a	3,5 ± 0,9 ^{ab}	3,5 ± 0,9 ^a
F ₉	3,8 ± 1,0 ^b	4,1 ± 0,7 ^b	3,7 ± 0,8 ^a	3,8 ± 0,8 ^b	3,8 ± 0,9 ^a
Average	3,6 ± 0,9	3,8 ± 0,9	3,7 ± 0,8	3,6 ± 0,8	3,7 ± 0,9 ^a
Reference	2,1 - 3,1 [*]	1,8 - 3,6 [*]	4,3 - 5,7 ^{**}	2,3 - 3,0 [*]	3,3 - 4,0 ^{***}

Note: Numbers followed by the same superscript letter indicate no significant difference; *Source (Nabilah, 2019); **Source (Nindyarani et al., 2011); ***Source (Setyadjid & Setiyaningrum, 2022)

CONCLUSION

The physicochemical properties, antioxidant activities, and sensory of cookies made from Mocaf flour and purple yam have been evaluated. The results of the physicochemical properties test showed that the cookies produced could not meet all the established quality standard criteria. However, in terms of antioxidant activity showed that the use of Mocaf flour, purple yam flour, and cinnamon powder can produce cookies with strong and very strong criteria of antioxidant activity. Formulations of F₈ and F₉ show the highest antioxidant activity (IC₅₀), which were around 49.5 ppm and 44.5 ppm, respectively (very strong category). The high composition of purple yam

flour and cinnamon powder tends to provide better physicochemical characteristics and antioxidant activity (IC₅₀) compared to the use of Mocaf flour only. The sensory results showed that F₂ was the most preferred cookie compared to other formulations. Further research is needed to get the right formulation to produce cookies with balanced physicochemical properties, antioxidant activity, and sensory.

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THE ROLE OF MINERAL AND SYNBIOTIC TO ENHANCE IMMUNITY DURING COVID-19 PANDEMIC : A LITERATURE REVIEW

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ABSTRACT

COVID-19 has become a pandemic in the last 3 years worldwide and cases cause high mortality and morbidity. To reduce COVID-19 infection, we need to keep our immune system healthy. Several nutrients have been shown to have specific abilities to increase the power of the immune system, but their use in the treatment of COVID-19 is still being debated. This review aims to determine the role of minerals and synbiotics in increasing immunity during the COVID-19 pandemic. Specific minerals such as zinc, selenium, iron and copper have promising potential to treat COVID-19 by reducing clinical impact, markers of inflammation, and improving immunological biomarkers. In addition to increasing mineral intake, maintaining a healthy immune system can also be done by improving the health of the gut microbiota. One of the therapies that is considered to have a positive impact on handling COVID-19 is using synbiotics (a combination of prebiotics and probiotics). However, the safety and efficacy of mineral and synbiotic supplementation in COVID-19 patients as adjunctive therapy still requires further research. Minerals and synbiotics can help boost the immune system and reduce symptoms during a COVID-19 infection.

Keywords: COVID-19; immunity; mineral; synbiotic; SARS-CoV-2

INTRODUCTION

Coronavirus Disease-19 (COVID-19) is an acute infectious respiratory disease transmitted through droplets and caused by an RNA virus called SARS-CoV-2. World Health Organization has announced that COVID-19 are globally pandemic after its outbreak in all countries. Indonesia itself has gone through third wave of COVID-19 cases with more than 6 million positive cases and 157 thousand death cases on 26 May 2022. COVID-19 specifically gives symptoms like fever, headache, difficulty of breathing, dyspnea, dry cough, vomit, diarrhea, and for several cases leave invasive lesion in the lungs (C. Huang et al., 2020; Shi et al., 2020). Severity of the COVID-19 cases are very diverse and depends on the occurrence of several comorbidities like cardiovascular disease, hypertension, metabolic syndrome, lung diseases, and diabetes mellitus type 2. The mortality also higher in older people and has at least one comorbidities (Mungroo et al., 2020; Zheng et al., 2020).

As it rapid transmission and evolution, until now there are no specific drugs that have been found to cure or prevent COVID-19 infection. So that it is important for people to always make sure their immune system in its best condition so that can fight COVID-19 without further symptom. Human immunity is a complex system that need several nutrients in order to make immune cells work optimally and can combat pathogenic agents (Shetty, 2010; Wood, 2006). One of which mineral that plays major role in providing better immune system, namely zinc, iron, selenium, and cooper. Several studies has showed information about the correlation between mineral and immune system both innate and adaptive (Gombart et al., 2020; Wintergerst et al., 2007). Deficiencies of several mineral known can affect immune cell function and make people more susceptible to infections (Calder et al., 2020; Gombart et al., 2020). So that it is important to make sure adequate intake of mineral during COVID-19 pandemic.

In order to gain an optimal immune system against COVID-19, it is necessary to also maintain

gut health. It is important because the SARS-CoV 2 can enter bloodstream through Angiotensin Converting Enzyme (ACE2) receptor which mostly can be found in digestive tract (Li et al., 2003; Zou et al., 2020). This also answer why many cases of COVID-19 has developed digestive symptom during infection period. Several studies has shown that the usage of synbiotic (combination of prebiotic and probiotic) can act as prevention to infections related to gut by helping balance intestinal microecology, improve the microbiota dysbiosis, and prevent secondary infections caused by bacterial translocation (Xu et al., 2020). But, the connection between synbiotic and COVID-19 still unclear and need further studies.

Reviewing the needs to explore the connection between mineral and synbiotic intake, it is necessary to make a review to investigate these correlation. In this review, we assessed the role of mineral and synbiotic in supporting the immune system and its correlation to COVID-19.

THE ROLE OF MINERAL IN THE IMMUNE SYSTEM

1. Zinc

Zinc takes significant part within the immune system. The free form of zinc has an immediate antiviral effect (Alpert, 2017). The daily requirement for zinc is 8-11 mg/day, with an upper limit of 40 mg/day. Zinc intake as many as 30-50 mg/day during infection is recommended to control RNA virus, such as influenza and coronavirus (Institute of Medicine, 2001; McCarty & DiNicolantonio, 2020). If zinc deficiency occurs, then there will be an increase in the risks of viral infection, thymic atrophy, lymphopenia, and decreased lymphocyte responses. Zinc can be found in lean red meat, whole grain cereals, nuts and legumes (Hidayati et al., 2019).

Zinc can inhibit enzymatic activities, SARS-CoV RNA polymerase replication, and inhibit ACE2 activities. Zn^{2+} also reduces the permeability of cell membranes without damaging nor penetrating the cells. Zinc provides protective effects in the prevention and COVID-19 therapy, where zinc increases the capillary epithelial barriers

and inhibit the transcapillary protein plasma movement. Therefore, zinc reduces local oedemic incidents, inflammation, exudation, and mucus secretion, preventing lung injury due to the use of a ventilator, modulating the antiviral immunity, and being a regulator of the tight junction of ZO-1 and Claudin-1 proteins to increase its barrier functions so that the virus can be prevented (Hunter et al., 2020; Skalny et al., 2020).

Zinc is vital for cell growth and differentiation of both innate and humoral immune cells, and also modulate cytokine release and trigger T cell $CD8^+$ proliferation (Wintergerst et al., 2007). Zinc is also vital for the intracellular binding of tyrosine kinase at T cell receptors, which is required for the development and activation of T lymphocytes (Wintergerst et al., 2006). Furthermore, zinc is a cofactor for 750 transcriptional factors for protein synthesis related to the immune and a cofactor for 200 enzymes involved in the formation of antioxidants, such as superoxide dismutase (SOD) and SMAD anti-inflammatory protein, by stabilizing the tertiary structure and being an essential component on the catalytic site of enzymes (Andreini et al., 2011; Gammoh & Rink, 2017). Zinc is needed in the production of the metallothionine antioxidant complex that is responsible for the lungs' elasticity. Moreover, it has been noticed that zinc plays a role in doubled-reducing the mortality rate due to pneumonia in people with adequate zinc intake (Barnett et al., 2010).

Zinc supplementation causes transient zinc chelation by N,N,N',N'-tetrakis(2-pyridinylmethyl)-1,2-ethanediamine (TPEN) to induct the antiviral inside cells through the activation of NF- κ B that triggers the interferon signaling. Zinc also roles as an anti-inflammatory agent, which triggers the development of Treg, Th17, and Th9 cells and helps the production of IgG antibody (Bonaventura et al., 2015; Gombart et al., 2020; Subramanian Vignesh & Deepe Jr, 2016). Zinc is a part of some antiviral compounds, namely zinc N-ethyl-N-phenyldithiocarbamate (EPDTC). Zn^{2+} ion also triggers viricidal activities by damaging the receptors on the surface of the viral cell through ions-centered tetrahedral geometric coordination that functions as an inhibitor against 3C and 3C-like proteases

(Lee et al., 2009). Zinc is also able to decrease the expressions of IL-6 plasma, IFN- α , IL-1b, and TNF- α genes. On the other hand, zinc can increase the IFN- α mediated by JAK1/STAT1 through signaling and increase antiviral enzyme, for instance, latent ribonuclease (RNase L) and protein kinase RNA (PKR), which results in RNA degradation and RNA translation inhibition (Günzel & Yu, 2013).

In vitro study revealed that zinc could reduce the ability of RNA replication by inhibiting RNA polymerase as in coronavirus (Martindale et al., 2020). The antiviral zinc-finger protein complex (ZAP) controls the process of virus entry, DNA/RNA replication, and the spread of viral infections (Wang et al., 2010). ZAP ACCHC3 can bind to RNA and facilitate intracellular RNA detection by activating retinoic acid-inducible gene-I (RIG-1)-like receptors (RLRs) and MDA5. The process then causes the kinases such as TBK1 and I κ B phosphorylates the interferon regulatory transcription factor 3 (IRF3) and I κ B- α (inhibitor of NK- κ B) that increases the type-1 interferon. IFN- α triggers the signal to escalate the antiviral protein (RNase L and PKR) that degrades and restrains the process of RNA translation. Zinc inhibit the NK- κ B activities using A20 (ZAP) protein expression that decreases TNF receptor regulation and initiates TLR-NK- κ B tracks. Zinc also acts as a cyclic nucleotide phosphodiesterase (PDE). When PDE is inhibited, it will increase cyclic nucleotide guanosin monophosphate (cGMP) which activates PKA (protein kinase A) and inhibits NK- κ B.

2. Iron

Iron is one of the essential nutrients for the body with various functions, including energy metabolism, growth and development, and the immune system (Sundari & Nuryanto, 2016). Iron can be found in numerous food sources, for instance, red meat, liver (beef and chicken), beans, red rice, and dark green leafy vegetables (spinach, kale, and others) (Calder, 2020). Iron is needed by the ribonucleotide reductase enzymes to synthesize the DNA, which functions to form lymphocyte-T cells. Iron deficiency can impaired the myeloperoxidase enzyme functions in the immune system (Sundari & Nuryanto, 2016).

Care needs to be taken in providing iron supplementation in people suffering from infectious diseases. Studies in tropical areas affirmed that iron administration to children with a dose above a certain threshold could escalate the risk of malaria and other infections, including pneumonia. Therefore, the intervention of iron in malaria-endemic areas is not recommended due to several reasons. First, excess iron may lead to the disruption of the immune functions. Second, excess iron can worsen the inflammation. Third, microorganisms need iron to support the growth of the pathogen (Cherayil, 2010; Drakesmith & Prentice, 2012; Ganz, 2018; Ganz & Nemeth, 2015; Nairz et al., 2017, 2018; Oppenheimer, 2001; Weiss, 2002).

Based on those reasons, hence, some methods have been developed to restrain iron-binding or used by pathogens. A study revealed that the provision of iron as much as 50 mg for four days in a week to school-aged children with iron deficiency increased the risk in respiratory tract infections. On the other hand, the addition of omega-3 PUFA as much as 500 mg for four days a week can decrease the adverse effects of iron supplementation (Malan et al., 2015). A meta-analysis study in Chinese children disclosed that those who undergo recurrent respiratory tract infections tend to be lack of iron on their hair (Mao et al., 2014). Thus, it can be implied that the administration of iron must be precise, whether the doses, the patient's condition, or the way of administering.

3. Selenium

Selenium was discovered by John Jakob Berzelius, a Swedish scientist, in 1817. According to Avery J.C. and Hoffman PR, selenium in the human immune system can be studied from the perspectives of immunobiology, leucocyte function increase, and the immune response towards pathogens and anti-cancers (Avery & Hoffmann, 2018). In general, seafood and internal organs are rich sources of selenium. In addition, meat, whole grains, dairy products, and eggs are also good sources of selenium (Kusmana, 2017).

Selenium deficiency can generate immune-incompetence, which will enlarge the risks of viral infections. Epidemiological study in China revealed the positive correlations between the

population selenium levels and COVID-19 recovery rates in 17 cities (Zhang et al., 2020); the higher the selenium level in the body, the faster the recovery of COVID-19 patients. Selenium is one of the micronutrients with essential roles in the immune system, particularly in suppressing the occurrence of oxidative stress. COVID-19 includes in viral infections related to the increase in oxidative stress by enhancing enzyme-producing ROS. Selenium, in the form of sodium selenite reduces the ROS production and the apoptosis of infected cells (Kretz-Remy & Arrigo, 2001).

RNA virus could be a trigger of NF- κ B (Nuclear Factor kappa B) activation. The activation of NF- κ B in cells infected with the nucleocapsid protein from the SARS-CoV can cause the severity of inflammation in lung lesions in SARS patients (Liao et al., 2005). Selenium has a role as the NF- κ B inhibitor among mice exposed to the SARS-CoV, which relates to the survival/immunity (DeDiego et al., 2014).

Besides the functions that have been elucidated, selenium also enhance the activity of the glutathione peroxidase (GSH-Px) (Ghneim, 2017). Selenium in the glutathione peroxidase acts as the catalysator in breaking down the peroxides to be a non-toxic/non-reactive bond. Together with vitamin E, selenium can protect endothelial cells/cell membranes that become the target of SARS-CoV-2 infection (Brigelius-Flohé et al., 2003). The integrity of cell membranes is fundamental, given the cytokine production is determined by the receptor in the cell membrane; hence, selenium is influential in increasing cellular immunity. Selenium is also an antioxidant that boosting the immune system. Selenium deficiency has a significant impact on the activity of selenoprotein antioxidant (specifically Gpx 1 expression) and on reducing the mRNA signal related to the inflammatory pathways. Thus, reducing the body's resistance against the viruses (Z. Huang et al., 2012).

4. Copper

Copper acts as the cofactor in the cellular metabolic reactions and copper-dependent enzymes catalyst reactions that involve molecular oxygen species. Several copper enzymes play a role in the

body's antioxidant defenses (Shetty, 2010). Copper is a micronutrient needed by pathogens and the host during the viral infection. Copper support Th cells, B cells, neutrophil, NK cells, and macrophage that influences the innate and adaptive immune responses (Raha et al., 2020). Copper also supports macrophage functions (copper accumulates in the phagolysosomes of macrophages to fight infectious agents), neutrophil, monocytes, and also increases the activity of NK cells. Furthermore, copper plays a role in the differentiation and proliferation of T cells, as a component of intrinsic antimicrobial which has anti-inflammatory action, antioxidant, and oxidative burst (Gombart et al., 2020). It is believed that copper has a role in the inflammatory responses given copper is a part of Cu/Zn SOD enzymes, which are the keys in the defense against ROS in maintaining the balance of intracellular antioxidant along with selenium and zinc (Gombart et al., 2020; Wintergerst et al., 2007).

The data regarding copper deficiency in humans is very limited due to lack of efficiency usages, homeostasis, and the appropriate parameters to determine the status of copper. The sufficient amount of copper intake enhanced the Th1 responses, decrease T cell proliferation, and increase B cell circulations. A high dose of copper intake (7 mg per day) for healthy adult males in an extended period can reduce the percentage of neutrophilic circulation, IL-2 serum receptor, and antibody titers against influenza virus strain Beijing. On the contrary, the same dose for the same subjects can increase the average immune responses (IL-6). Moreover, there is a pro-oxidant effect that makes this high dose of intake protect red blood cells against peroxidation induced in vitro (Wintergerst et al., 2007).

Copper can kill certain contagious viruses, such as bronchitis virus, poliovirus, HIV type 1, both enveloped and nonenveloped viruses, and single or double-stranded DNA and RNA viruses. Thus, the addition of copper intake can encourage both the innate and adaptive immune systems (Raha et al., 2020). However, until recently, the registered trials to disclose the impact of copper supplementation on COVID-19 patients is not yet published.

THE ROLE OF SYNBIOTIC IN THE IMMUNE SYSTEM

The use of synbiotic (combination of prebiotic and probiotic) in preventing the risks of infections began to be noticed. The Zhejiang Hospital of China recommended the provision of synbiotic in COVID-19 patients to help balance intestinal microecology, improve the microbiota dysbiosis, and prevent secondary infections caused by bacterial translocation (Xu et al., 2020).

1. Probiotics

Some studies have revealed the effects of probiotics (*Bifidobacterium* and *Lactobacillus*) provisions in reducing respiratory infections (de Araujo et al., 2015; Ichinohe et al., 2011). Probiotics can escalate the interferon and the number and activities of antigen, NK cells, T cells, as well as specific antibody both systemic and mucosal (Namba et al., 2010; Zelaya et al., 2016). Probiotics are proven influential in regulating pro-inflammatory and immunoregulatory cytokines that control the clearance virus and prevents lung damages caused by the immune responses. *Lactobacillus plantarum* DR7 is affirmed to be able to suppress the proinflammatory plasma cytokines (IFN-gamma and TNF-alpha), increasing the anti-inflammatory cytokines (IL-4, IL-10), and decreasing the plasma peroxides and the oxidative stress (Chong et al., 2019). It is important in COVID-19 patients which experiencing the cytokine storm. Probiotics can also enhance the tight junction integrity and production of the short chain fatty acid (SCFA) Butyrate, and provide nutrition for colonocytes thus, reduce the SARS-CoV-2 invasion (Baud et al., 2020). Studies also found that probiotics could upsurge the amount of leucocyte, neutrophil, IL-2, TNF-beta, decrease the cytokine expressions (TNF-alpha, IL-1beta, IL-6, IL-8, IL-5, IL13), and IgA saliva level can produce bacteriocin and reuterin, promote phagocytosis, and can maintain Th1 and Th2 homeostasis (Fooks & Gibson, 2002; Guillemard et al., 2010).

Lactobacillus plantarum, as one type of probiotics has been shown to have antiviral activities against coronavirus in the intestinal epithelial cells. *L.plantarum* can also provide IFN- λ 3 to suppress the enteric coronavirus infection and can be used as an alternative antiviral therapy

(Liu et al., 2020). Several meta-analyses showed the presence of probiotic effects (*Lactobacillus rhamnosus* GG, *Bacillus subtilis*, and *Enterococcus faecalis*) in decreasing the incidence and the viral infection duration of the critically ill patients with respiratory tract infections (Hao et al., 2015; King et al., 2014). Xu et al., in their study, concluded that many COVID-19 patients in China experience dysbiosis of intestinal microbiota, which is marked by the decrease in *Lactobacillus* and *Bifidobacterium* because the use of antibiotics and COVID-19 causes diarrhea (Xu et al., 2020).

Probiotics, such as *Lactobacillus plantarum*, *Lactobacillus casei*, *Bifidobacterium animalis*, *Bacillus coagulans*, *Streptococcus salivarius*, and *Enterococcus faecium* have proinflammatory interleukin inhibitor effects. On the other hand, *Lactobacillus gasseri*, *Lactobacillus rhamnosus*, and *Bifidobacterium longum* are acknowledged for their ability to increase the antibody. *Bifidobacterium animalis* can prevent the coronavirus replication by lowering the inositol-requiring enzyme 1 (IRE1) pathway, thereby reducing interleukin 17 (Bozkurt et al., 2019). *Lactococcus lactis* JCM5805 activates plasmacytoid dendritic cells (pDC), where the pDC acts as the cells that produce IFN1 (Siegal et al., 1999; Trinchieri & Santoli, 1978) and mucosal T cells (Tezuka et al., 2011). Moreover, pDC can directly prevent viral spread and replication (Theofilopoulos et al., 2004), and activate the NK cells (Tezuka et al., 2011). Additionally, some probiotics, for instance, *Enterococcus faecium* HDRsEf1, can reduce the mRNA TLR4, TLR5, TLR7, and TLR8 (Tian et al., 2016).

Probiotics in Indonesian foods can be found from sayur asin, tempoyak, mandai, tape, growol tempe, kecap, bakasang, dadih, and many more. Mostly these foods are rich in lactic acid bacteria that good to our health (Nuraida, 2015).

2. Prebiotics

Prebiotics, which are undigested carbohydrates such as inulin, polydextrose, oligosaccharides, fiber, and resistant starch, are used by intestinal microbes for fermentation. Prebiotics are also acknowledged to increase the immunity and the diversity of the gut microbiota, as well as aiding digestion (Bouhnik et al., 2007). As an example,

prebiotics obtained from wheat is proven to reduce the proinflammatory cytokine IL-6 and to boost the anti-inflammatory cytokine IL-10 (Keim & Martin, 2014; West et al., 2017). Prebiotics such as wheat bran, fructooligosaccharides (FOS), and galactosaccharides (GOS) can increase the butyrate levels that reduce inflammation and improve the respiratory fibrosis (Anand & Mande, 2018). SCFA from prebiotic metabolism strengthens the gastrointestinal association with lymphoid tissue (FALT) (Schley & Field, 2002). Hence, administering prebiotics and probiotics to COVID-19 patients can help to improve the intestinal dysbiosis conditions, thereby accelerating the healing process. Prebiotics can help fight respiratory infections as proven by Trompette et al. in their research, where the subject mice fed with prebiotic dietary fiber experienced an increase in macrophage and a reduction in the production of chemokine CXCL1, which causes neutrophil increases in the lungs, as well as adding the CD8⁺ cell functions (Trompette et al., 2018).

An RCT involving 94 premature babies revealed that the intervention of mixed prebiotic galactooligosaccharide and polydextrose (1:1) or probiotic *Lactobacillus rhamnosus GG* reduces the incidence of respiratory tract infections by 2-30 times compared to placebo (Guillemard et al., 2010). Additionally, the administrations of synbiotic *Pediococcus pentosaceus* 5-33:3, *Leuconostoc mesenteroides* 32-77:1, *L. paracasei ssp. paracasei* 19, *L. plantarum* 2,362 in conjunction with inulin, oat bran, pectin, and resistant starch in critically ill patients with a ventilator are proven to decrease the rates of infections, sepsis, SIRS, length of treatment, the period of using a ventilator, and mortality (Kotzampassi et al., 2006).

In Indonesia, prebiotic are mostly can be found in tuber crops, like gembili, yam, dahlia root, potato, sweet potato, and cassava. Prebiotic also can be found in chicory, artichoke, and garlic (Zubaidah & Akhadiana, 2013).

CONCLUSION

Enhancing immune system during COVID-19 pandemic is necessary. The use of zinc during COVID-19 infection can give better result of treatment. Minerals with anti-inflammatory

and antioxidant properties can help to reduce inflammatory response during COVID-19 infection. The usage of synbiotic also can help enhance immune system by balancing intestinal microecology and microbiota balance so it can help preventing the infection of COVID-19. However, the safety and efficacy of nutritional supplementation, including minerals and synbiotic as adjunctive therapy for COVID-19 patient needs further studies.

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STUDY OF SATISFACTION TOWARDS THE FOOD SERVICES IN THE PPLP AND SKO ATHLETE DORMITORIES IN INDONESIA

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ABSTRACT

Providing food for athletes is a special institutional arrangement that caters to athletes' specific needs. Athletes have different nutritional requirements than the non-athlete population, as they need more nutrients to compensate for the energy expended during training, competitions, and recovery after matches. Additionally, athletes must focus on fluid intake and sufficient nutrients to support post-training recovery for optimal performance. Therefore, meeting the needs and satisfaction of athletes becomes a crucial goal for athlete food service. Satisfaction can be assessed based on various indicators such as variety of menu and ingredients, suitable portion size, taste, serving time, packaging, and food handler hygiene. This study aims to assess athletes' satisfaction with the food service in athlete dormitories. The research is an observational survey with a cross-sectional approach involving 159 athletes aged 13-19 years from PPLP West Java, PPLP DIY, PPLP NTB, and SKO Cibubur. Data collection took place in May and July 2022. The research instrument used was a customer satisfaction survey questionnaire with a Likert scale ranging from 1 to 4, where one indicates 'very poor' and four indicates 'excellent.' The analysis technique used Pearson correlation in SPSS 16.0 software. The research showed a relationship between menu variety, ingredient variety, suitable portion size, serving time accuracy, food packaging, serving area, food handler hygiene, food presentation, and food taste with athletes' satisfaction level. Serving time accuracy, food packaging, hygiene, cleanliness of the serving area, food handler hygiene, food appearance, and taste correlate strongly with satisfaction.

Keywords: food service, athlete, satisfactory study, athlete dormitory

INTRODUCTION

The provision of meals includes a series of activities ranging from menu planning to food presentation and distribution (Widyastuti et al., 2018; Penggali et al., 2021). Based on the type of institution, meal provision for athletes falls under the category of meal provision in specialized institutions because athletes have different needs compared to the general population and need to consider other aspects, such as training

periodization and caloric requirements based on the type of sport (Widyastuti et al., 2018; Penggali et al., 2021). Athlete meal provision is one of the supportive aspects aimed at providing high-quality food for athletes to support their optimal health, which is crucial for enhancing athletic performance (Penggali et al., 2021).

Customer satisfaction is an assessment that aligns with customer expectations regarding various aspects of food service, including taste, cleanliness, healthiness, and nutritional value

(Widyastuti et al., 2018). Various cooking methods can affect the content of active ingredients and nutritional value of the food, as well as the appearance of the food, which can ultimately affect the acceptance of the food (Gliszczynska-Swig et al., 2006; Miglio et al., 2008). The variety of food ingredients and menu preparations also affects athletes' acceptance and satisfaction with the food served. One study by Afriani (2017) on pencak silat athletes in PPLP Yogyakarta showed that athletes' acceptance and satisfaction with staple foods were lower than animal protein, plant-based protein, vegetables, and fruits (Afriani et al., 2017).

Poor assessment of food quality can affect consumers' food intake. If consumers are not satisfied with the food served, it can lead them not to finish their meals or not consume the food. It affects the quantity and quality of nutrients entering the consumers' bodies. Previous research conducted by Marlenywati (2017) at an integrated Islamic school found that 80% of students had nutrient intake below the Recommended Dietary Allowance for 2013, 70% of students were dissatisfied with the food served because it was not appealing, and 80% were dissatisfied because the food lacked variety (Marlenywati et al., 2017).

Nutrient intake is crucial to support athletes' performance, especially during adolescence when optimal physical growth occurs, requiring an adequate intake of nutrients (Penggalih et al., 2021; Marlenywati et al., 2017). Based on the Nutritional Adequacy Rate in 2019, adolescents aged 15-18 years have a carbohydrate requirement of 292 grams per day, and their calcium, iron, zinc, and magnesium needs are twice as high as other age groups (Permenkes, 2019; Almatier, 2011). These requirements increase further with the additional physical activity burden of athletes. Given this background, a satisfaction study on meal provision at athlete training centres and schools is important.

METHODS

The research in this study is an observational survey with a cross-sectional design. The research was conducted on adolescent athletes aged 13-19 years who trained at the PPLP (Center for Education and Sports Training for Students) in

West Java, DIY, NTB, and SKO (Special School for Athletes) in Cibubur, with a total of 159 subjects. The sampling technique in this study used total sampling by involving all athletes who received food services at the targeted PPLP and SKO institutions and were willing to provide assessments of meal provision. The instrument used was a meal provision satisfaction survey questionnaire conducted in May 2022 at these institutions. The survey questionnaire contained questions related to various aspects of menu variety, types of food ingredients, portion suitability, timeliness, packaging cleanliness, personnel, facilities, and food taste, rated on a Likert scale with scores of 1-4. A score of 1 is considered poor, 2 is considered sufficient, 3 is considered good, and 4 is considered excellent.

After data collection, descriptive analysis tests were conducted to determine the characteristics of the subjects, and Pearson correlation tests were conducted to determine the relationship between each assessment aspect and athlete satisfaction levels. Statistical analysis was performed using SPSS software version 16.0. This research has obtained ethical approval from the Faculty of Public Health and Nursing Ethics Committee numbers KE/FK/0100/EC/2021 with amandemen number was KE/FK/0512/EC.

RESULTS

The age of the research subjects was predominantly in the 13-16 years range, with an average age of 16 years, corresponding to grades X and XI. Regarding gender, more male athletes were participating in the study (Table 1).

Based on testing the relationship between various aspects of satisfaction assessment and the level of athlete satisfaction, the results show that the level of athlete satisfaction is considered sufficient, with an average score of 2.91.

This level of satisfaction is influenced by menu variety, variety of food ingredients, portion suitability for athletes' needs, timeliness of service, presentation packaging, cleanliness and tidiness of the serving area, cleanliness and tidiness of personnel, food appearance, and food taste. The variable with the most robust relationship with the level of satisfaction, as assessed by

Table 1. Characteristic of the participants

Variable	N (%)
Age	
Early adolescent (13-16)	93 (58,4%)
Late adolescent (17-19)	66 (41,6%)
Gender	
Male	81 (50,9%)
Female	78 (49,1%)

Table 2. Relationship between various assessment aspects and satisfaction level

Variable	r	Satisfaction level
		p-value
Punctuality in serving	1**	0,000*
Neat and clean food packaging	1**	0,000*
Clean and orderly presentation area	1**	0,000*
The serving staff maintains cleanliness	1**	0,000*
The appearance of the served food	1**	0,000*
Taste of the food	1**	0,000*
Overall menu variation	0,832**	0,000*
Menu variation of carbohydrate	0,812**	0,000*
Menu variation of animal protein	0,782**	0,000*
Menu variation of plant protein	0,764**	0,000*
Menu variation of vegetable	0,781**	0,000*
Variation of carbohydrate	0,817**	0,000*
Variation of animal protein	0,819**	0,000*
Variation of plant protein	0,837**	0,000*
Variation of vegetable	0,816**	0,000*
Variation of fruit	0,694**	0,000*
The suitability of carbohydrate dish portions to meet the needs	0,851**	0,000*
The suitability of animal-based dish portions to meet the needs	0,825**	0,000*
The suitability of plant-based dish portions to meet the needs	0,877**	0,000*
The suitability of vegetable dish portions to meet the needs	0,781**	0,000*
The suitability of fruit portions to meet the needs	0,805**	0,000*

* significant if $p \leq 0.05$;** there is an association if $r > 0,159$ (r table)

the Pearson correlation test, is the timeliness of service, followed by food packaging, cleanliness and tidiness of the serving area, cleanliness of personnel, food appearance, and food taste (Table 2). The better the service aspects in terms of timeliness of service, neat and clean food packaging, cleanliness and tidiness of the serving area, cleanliness of personnel, and food taste, the higher the level of satisfaction will be.

The assessment of athletes on several aspects of satisfaction in the provision of meals at PPLP DIY, West Java, NTB, and SKO Cibubur is considered satisfactory, with an average score above 2.5 to 3.0 out of a total score of 4.0. This indicates that the majority of athletes have given a positive assessment of the meal provisions at these locations. Therefore, it can be assumed that the meal provisions at these places generally meet the expectations or satisfaction of the athletes in various measured aspects. However, it is still important to continuously monitor and improve the quality of food services to ensure the athletes' satisfaction is consistently maintained.

Regarding menu variety, the average score ranges from 2.7 to 3.0, which falls into the categories of fair to good (Table 3).

Regarding the variety of food ingredient groups processed, the average assessment score ranges from 2.69 to 2.94, which falls into the category of "satisfactory" (Table 4).

Regarding the appropriateness of portion sizes for each dish group, the assessments range from 2.79 to 2.97, which falls into the "satisfactory" category (Table 5).

A good rating with an average score of 3.1 is given to aspects related to timeliness, cleanliness, and the neatness of food packaging, the dining

Table 3. Assessment of menu variation aspect

Variable	N	Mean
		± Std. Deviation
Overall menu variation	159	2,89 ± 0,83
Menu variation of carbohydrate	159	3,03 ± 0,76
Menu variation of animal protein	159	2,94 ± 0,83
Menu variation of plant protein	159	2,79 ± 0,77
Menu variation of vegetable	159	2,7 ± 0,89

Table 4. Assessment of aspects of variations in types of food ingredients

Variable	N	Mean
		± Std. Deviation
Variety of carbohydrate	159	2,94 ± 0,75
Variety of animal protein	159	2,86 ± 0,81
Variety of plant protein	159	2,79 ± 0,76
Variety of vegetable	159	2,69 ± 0,87
Variety of fruit	159	2,86 ± 0,89

Table 5. Assessment of portion suitability aspect

Variable	N	Mean
		± Std. Deviation
The suitability of carbohydrate dish portions to meet the needs	159	2,97 ± 0,72
The suitability of animal-based dish portions to meet the needs	159	2,91 ± 0,72
The suitability of plant-based dish portions to meet the needs	159	2,79 ± 0,79
The suitability of vegetable dish portions to meet the needs	159	2,92 ± 0,81
The suitability of fruit portions to meet the needs	159	2,89 ± 0,89

Table 6. Assessment of aspects related to timeliness, packaging, premises, personnel, presentation, and taste of food

Variable	N	Mean
		± Std. Deviation
Punctuality in serving	159	3,18 ± 0,75
Neat and clean food packaging	159	3,11 ± 0,79
Clean and orderly presentation area	159	3,17 ± 0,73
The serving staff maintains cleanliness	159	3,16 ± 0,77
The appearance of the served food	159	2,83 ± 0,88
Taste of the food	159	2,82 ± 0,85

area, and the staff. Meanwhile, other aspects are considered satisfactory, ranging from 2.82 to 2.83.

DISCUSSION

a. Menu variation

Good food quality enhances athletes' perception of food satisfaction, increasing their attendance in the cafeteria and reducing the likelihood of athletes purchasing food outside the cafeteria (Jamaluddin et al., 2014).

Based on the assessment of 159 athletes, the evaluation of menu variety in the food provision at PPLP NTB, DIY, West Java, and SKO Cibubur tends to be good, with an average score of 2.89 out of a total score of 4 (Table 3). The menu variety that received the highest rating is the carbohydrate menu, with a score of 3.03, followed by the animal protein menu with 2.94, the vegetable menu with 2.79, and the variety of vegetables with 2.7 (Table 3). It can be seen from the reasonably diverse menu options. The carbohydrate menu has options such as white rice, wet rice, yellow rice, fried rice, ulam rice, *daun jeruk rice*, fried vermicelli, fried noodles, and spaghetti bolognese. In the animal protein menu, meat, chicken, eggs, and fish are prepared into various dishes, including hot and sour cook soy sauce, *opor*, *padang* spices, *curry*, *tongseng*, *bali* spices, *rawon*. The vegetable menu has various preparations of tempeh, tofu, and legumes, which are cooked as stir-fry, fritters, dreadlocks, steamed sugar, soup, and fried. The vegetable menu variety consists of stir fry, soup with condiment, soup with coconut milk, stew, sautee.

The data indicates that menu variety, both overall and specific to each menu category (carbohydrates, animal protein, plant-based protein, vegetables), is significantly related to athletes' satisfaction levels in food provision. Research conducted by Baiomy et al. (2017) states that factors influencing consumer satisfaction in food provision include menu descriptions, variety, and design (Jawabreh et al., 2018).

a. Type variation

The variety of food ingredients served (such as carbohydrates, animal protein, plant-based protein, vegetables, and fruits) has been shown to influence athletes' satisfaction levels with food provision significantly. This data aligns with research conducted by Ismail et al. (2019), which stated that with the increasing awareness of consumers in collage cafeteria that there is no single type of food could fulfil all nutritional needs. The availability of various types of

food ingredients (such as fruits, vegetables, carbohydrates, protein, and fats) can attract consumers to dine in the cafeteria (Ismail et al., 2019).

The athletes' assessments of the variety of food ingredients in the food provision show scores from highest to lowest: 2.94 for carbohydrates, 2.86 for animal protein, 2.86 for fruits, 2.79 for plant-based protein, and 2.69 for vegetables (Table 4). Overall, the assessment of the types of food ingredients processed is considered quite good due to the variety in the sources of carbohydrates, including rice, bread, noodles, pasta, potatoes, and flour-based products. The animal protein category combines various ingredients, including chicken, beef, lamb, chicken liver, chicken eggs, duck eggs, freshwater fish, sea fish, squid, shrimp, meat rolls, and fish meatballs. Various types of fruits, such as oranges, snakefruit, melons, watermelons, bananas, and papayas, show good variation. For plant-based protein, the food provision at PPLP and SKO processes various legumes, tempeh, and tofu. Likewise, with vegetables, there are various types, including cabbage, water spinach, spinach, carrots, cauliflower, green beans, long beans, broccoli, corn, green onions, mustard greens, white mustard greens, tomatoes, and bean sprouts.

In PPLP West Java, based on a 6-day cycle, the most frequently appearing type of carbohydrate is white rice with a frequency of 18 times, followed by potatoes 1 time, noodles 1 time, and vermicelli 2 times. The most frequently provided animal protein is chicken, with a frequency of 7 times, followed by fish and eggs 6 times, beef, tofu, and tempeh each 3 times, meat rolls 1 time, and chicken gizzards 1 time. Mixed carrots and cabbage appear most frequently among vegetables, with a frequency of 6 times, followed by green beans, long beans, green mustard, white mustard, red beans, chayote squash, jackfruit, and mushrooms, each appearing once. Fruits are served alternately daily, including melon, banana, orange, papaya, and watermelon.

RAMU	RAMU	RAMU
NASI PUTIH	NASI PUTIH	NASI PUTIH
TONGKOL, SARDEN	KEMAP, SARDEN, PANGKANG	AYAM, KEMAP
TELUR, SPINACH	KEMAS, TELUR	STUP, SPINACH
KEMAPUK	PERKEBES, TEMPE	ORANG, TELUR
TELUR, BUNYAN	ORUPUK	ORUPUK
AYAM, BUNYAN	BUNYAN, BUNYAN	BUNYAN, BUNYAN
AYAM, BUNYAN	AYAM, BUNYAN	AYAM, BUNYAN

Figure 1. Example of a one-day menu cycle at one of the PPLP facilities

\ The significant relationship between the variety of food ingredients and customer satisfaction is consistent with research conducted by Tanuwijaya et al. (2019), which showed that the greater the variety of food ingredients used, the higher the level of satisfaction. Furthermore, as the variety of food ingredients increases, the menu options also expand. Consequently, customer satisfaction can increase with a more diverse menu selection.

b. Portion Size

Portion adequacy is one of the crucial aspects of food provision that can influence consumer satisfaction (Agustina, 2016). Standardized portions can be used to predict nutrient adequacy and consumer intake. The amount of food portions athletes need can vary between different types of sports (sports disciplines). For example, endurance sports may require higher calorie intake due to the longer duration of exercise, ranging from 30 minutes to 4 hours, while strength sports generally have shorter exercise durations. Therefore, the quantity and type of carbohydrates needed may differ. In endurance sports, complex carbohydrates are needed in larger quantities than in strength sports, which require carbohydrates in simpler forms (Penggali, 2020). Portion sizes can also vary among individuals based on their preferences, affecting their satisfaction levels (Agustina, 2016). The size of food portions can also impact the visual presentation or appearance of the food, affecting their likability.

The food portions' suitability received a good rating with an average score of 2.79 to 2.97 out of a total score of 4. It indicates that athletes are satisfied with the standardized portions of food served for carbohydrate variants, animal protein, plant-based protein, vegetables, and fruits. This study suggests that portion suitability correlates with athletes' food provision satisfaction. Research on food provision for athletes at PPLP West Java conducted by Putra (2021) also shows a relationship between portion suitability and athlete satisfaction.

Taste, Food Appearance, Presentation Time, Cleanliness of Space, and Service

Providing food specifically for athletes should aim to meet their nutritional needs following the periodization of their training programs (Sedyanti,

2014). Among various assessment aspects, the appearance of food plays a crucial role in athlete satisfaction. Food appearance encompasses several components: presentation, portion size, texture, shape, and colour. The more diverse the food variations across these components, the more attractive it is to consumers (Putra et al., 2021). Additionally, good food appearance must be complemented by delicious taste. Unpleasant taste can diminish food quality and lead to food waste as consumer expectations are unmet (Heikkilä et al., 2016).

In the assessment of food taste, a score of 2.82 out of a total score of 4.0 was obtained (Table 6), indicating that athletes are reasonably satisfied with the taste of the food served. For the aspect of food appearance, the assessment score is 2.83 out of 4.0 (Table 6), indicating a “Satisfactory” level of satisfaction. Meanwhile, for the aspects of serving time, cleanliness of the dining area, and handling of food, scores above 3.0 were obtained, indicating a “Good” rating.

In this study, the accuracy of food serving time is closely related to consumer satisfaction both before and after the nutritionist intervention ($p=0.000$). According to research conducted (Sunarya & Puspita, 2018) on patients at the Sultan Syarif Mohamad Alqadri Hospital in Pontianak, timely meal service can enhance patient acceptability. Timeliness is also important to prevent food waste. Because the timing of meals matches meal times, consumers’ appetites remain intact. The food serving time accuracy also reflects the food service staff’s ability to adjust portion sizes and food composition to match consumers’ meal times (Dewi, 2019). The timing of meals for athletes needs to consider their training schedules. Based on research conducted by (Hasbullah et al., 2017), athletes are typically provided with 3 main meals and 3 snacks, with breakfast and dinner given after athletes finish their training to avoid digestive problems and optimize performance by providing a snack before training. Thus, training can be carried out optimally.

Applying hygiene and sanitation in food provision is essential to prevent food poisoning (Odeyemi et al., 2019). This study shows that consumer satisfaction is influenced by the cleanliness and tidiness of food handlers and

dining areas ($p=0.000$). This is in line with the research conducted by (2007), which found that the cleanliness of food service facilities and food handlers is a significant factor affecting consumers’ decisions to dine at a particular location. Most consumers rely on their judgment to assess hygiene risks, with the cleanliness of food handlers’ hands during food service being the primary assessment frequently conducted by consumers. Additionally, a study by Park et al. (2016) revealed that 5 sanitation dimensions affect consumers’ emotions or feelings towards consuming food at a food service facility: cleanliness of food handlers, dining area, food handling, dining room appearance, and the availability of bathrooms. Therefore, improving the knowledge and behaviour of hygiene in all aspects of food service is an important component, and regular programs should be implemented (Pepple, 2017).

Consumer satisfaction perceptions are also influenced by food packaging. In addition to food storage, packaging also serves as a product representation, maintains the safety and freshness of food, provides nutritional value information, and protects food products during distribution (Claudio, 2012). In the context of food provision at athlete training centres, self-service or buffet-style dining is commonly encountered. This type of service allows athletes to select various menu options provided in large serving containers (Penggalih et al., 2021). To enhance consumer satisfaction, the accuracy of food packaging should be considered, considering aspects such as the cleanliness of serving utensils, food temperature control, and alignment with athletes’ meal needs (Penggalih et al., 2021) (Rodgers, 2007).

CONCLUSION

The satisfaction rating of athletes at PPLP and SKO regarding the food provision in the athlete dormitories is considered good in several aspects, including the timely serving of meals and the cleanliness and neatness in food packaging, the dining area, and the food handlers. All assessed aspects related to menu variety in PPLP and SKO, both overall and for each type of dish, the variety of food ingredients, portion suitability for athlete needs, meal serving timeliness, packaging

presentation, dining area condition, food handler cleanliness, food appearance, and food taste, are associated with athlete satisfaction. The aspects that have the strongest correlation with satisfaction levels are the timeliness of meal service, food packaging, the cleanliness and tidiness of the dining area, the cleanliness of food handlers, food presentation, and food taste.

SUGGESTION

The correlation between food ingredient variety, menu variety, and athlete satisfaction in meal provision provides a basis for considering ongoing efforts to modify menus and arrange menu cycles to prevent athletes from becoming bored with the food provided. Menu cycles could be extended to longer, such as a 10-day cycle, to offer greater diversity in food options.

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FOOD WASTE AND FOOD SERVICE SATISFACTION AMONG OLDER ADULTS IN NURSING HOMES

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ABSTRACT

Food waste and food service satisfaction can be used of a good food service management. Nursing homes are an institution that also provides food service. This study aims to identify and analyze the association between food waste and food service satisfaction among older adults in the nursing home in Surabaya, Indonesia. A cross-sectional study was conducted on 63 older adults. The 3x24-hour visual Comstock method was used to identify food waste and food service satisfaction was assessed using a questionnaire adapted from the RFSQ (Resident Foodservice Satisfaction Questionnaire) and FoodEx-LTC (Food Expectations-Long-Term Care). Data were analyzed descriptively and inferentially using the Chi-square test. The association between food waste and older adults' food service satisfaction is insignificant ($p=0.115$). The majority of the older adults (71.4%) were satisfied with the food service and the average of food waste was relatively low ($\leq 20\%$). However, some menus still had $>20\%$ leftovers. It is important to investigate older adults' acceptability and preferences of each menu to minimalize food waste. Menu cycle planning needs to be evaluated periodically and well-designed to meet the nutritional needs of the residents.

Keywords: *diet, food service satisfaction, food waste, nursing home, older adults*

INTRODUCTION

Food service is a series of processes starting from menu planning, purchasing, preparation, processing, and distribution, to serving food till it is ready to be consumed by consumers (Andrini, 2012; Taqhi, 2014). Food service applied in nursing homes is non beneficially oriented that intended to meet the nutritional needs of the older adults in nursing home (Widyastuti et al., 2018). Therefore, the implementation of food service in nursing homes are prone to causing dissatisfaction.

Nursing homes are one of the government programs that are made to support the welfare of the homeless, poor, neglected, or living-alone older adults (BPS, 2021; Rahayu & Ma'ruf, 2018). Several previous studies have stated that older adults who live in nursing homes tend to be malnourished, have a lower BMI, and more likely to experience malnutrition than the older adults who live in the community (Saghafi-Asl & Vaghef-Mehrabany, 2017). Malnutrition is a condition that is often found in older adults (Amarya et al., 2015, 2018). Malnutrition have a higher risk of reduced

mobility, increased risk of falls and fractures, susceptibility to infectious diseases, and in the end, it can worsen the condition of malnutrition (Divert et al., 2015). Therefore, improving food service satisfaction in nursing homes are essential.

Nutritional problems in the older adults are closely related to inadequate intake of nutrients (Divert et al., 2015). Food service satisfaction and food waste are the indicators that can be used to assess food service successes as well as assess intake by assessing the estimated amount of intake (Puspa et al., 2019; Semedi et al., 2013). Adequate nutritional intake in the older adults is important due to its related to minimizing worsening of health and nutritional problems (Sofia & Gusti, 2017).

Food service satisfaction is known to be associated with improved nutritional status related to a more adequate amount of intake and minimal food waste (Semedi et al., 2013). Previous studies showed that the older adults care was institution that had the highest amount of leftover food (20.6%) after canteens, restaurants, and pre-school food service. And also, nursing home had the

highest number of leftovers per portions after restaurants and hotels, which amounted to 129 grams of leftovers per meal (Malefors et al., 2019). This shows that food waste in institutions such as nursing homes need to be considered and related to the food services provided. Therefore, this study aims to analyze the relationship between the level of satisfaction with food service and food waste for the older adults at nursing home in Surabaya.

METHODS

This observational study with a cross-sectional design was followed by the older adults living at nursing home in Surabaya. Population of older adults living at nursing home in Surabaya are 171 including independent, partial, and bed-rest older adults. The sample size obtained is 63 older adults calculated using Lemeshow formula. The sampling method was carried out using the simple random sampling method and justify based on central limit theorem. The sample inclusion criteria was the older adults at least 60 years old, had lived at nursing home in Surabaya for at least 3 months, independent older adults, able to communicate well, cooperative, and did not have memory disorders. The exclusion criteria for this study were the older adults with dementia and the older adults on bed rest.

This research was conducted from 2021 to 2022. The data collected consist of the characteristics of the older adults, food waste, and food service satisfaction. Characteristics data were obtained from older adults and nursing home's database including sex, age, length of stay, education level, medical history, and nutritional status based on BMI. Education level was classified as very low (did not go to school), low (primary or junior high school), moderate (senior high school), and high (college). While BMI was classified refers to Indonesian Ministry of Health classification which are underweight (<18.5 kg/m²), normal (18.5-25.0 kg/m²), and overweight/obese (>25.0 kg/m²) (Kemenkes RI, 2014).

Food waste assessment was carried out with the visual comstock method through observations made by nutritionists and food service satisfaction was carried out using interviews based on questionnaires. Food waste observations were

carried out for three days including breakfast, lunch, afternoon snack, dinner, and evening snack using a 0-5 scale (from 0% to 100% food waste) (BPPSDMK, 2018). Refers to Decree of Ministry of Health number 129/Menkes/SK/II/2008, food waste $\leq 20\%$ can be used as success food services indicator (Dewi, 2015).

The food service satisfaction questionnaire was designed by the references of the RFSQ (Resident Foodservice Satisfaction Questionnaire) by Wright et al. (Wright et al., 2008) and FoodEx-LTC (Food Expectations-Long-Term Care) by Crogan et al. (Crogan et al., 2004). The RFSQ and Food-Ex questionnaires were chosen because they were designed to assess food service satisfaction in nursing home settings. In addition, that questionnaires has been tested in several previous studies.

The data obtained were then analyzed descriptively and inferential analysis using the chi square test. This research has been approved by the Ethics Committee of the Faculty of Public Health, Airlangga University with the number 78/EA/KEPK/2022.

RESULTS AND DISCUSSIONS

A total of 63 older adults people participated in this study. Respondents were dominated by women (69.8%) with an age range of 60-74 years (61.9%). The length of stay of the older adults at nursing home in Surabaya is mostly 12-36 months. The nutritional status of the older adults based on Body Mass Index (BMI) showed that 15.9% were classified as underweight, although the normal nutritional status was recorded at around 50.8%. The characteristics of the older adults can be found in more detail at table 1.

In a food service system, leftovers can be used to assess the estimated amount of food intake. Food waste is influenced by several factors, including external and internal factors. External factors include taste, food quality, limited menu choices, inappropriate portion size and meal times, dining environment, economic, educational, and socio-cultural factors. While the internal factors that influence include appetite, eating habits, age, gender, and illness (Puspa et al., 2019; Simzari et al., 2017).

Table 1. Participant's Characteristics

Variable	n (%)
Sex	
Male	19 (30.2)
Female	44 (69.8)
Age (year)	
60-74	39 (61.9)
74-90	24 (38.1)
>90	0 (0.0)
Length of stay (month)	
<12	19 (30.2)
12-36	28 (44.4)
≥36-60	15 (23.8)
≥60	1 (1.6)
Education level	
Very low	17 (27.0)
Low	38 (60.3)
Moderate	6 (9.5)
High	2 (3.2)
Medical history	
Hypertension	46 (73.0)
Gout/arthritis	17 (27.0)
Diabetes mellitus	12 (19.0)
Stroke	4 (6.3)
Heart disease	1 (1.6)
Other	15 (23.8)
Nutritional status	
Underweight	10 (15.9)
Normal	32 (50.8)
Overweight/obese	21 (33.3)

In this study, the leftovers for three days are relatively small, but in certain menus, the leftovers are still quite high. A lot of leftover food can indicate that less food is consumed. Some respondents said the reason for leaving food was because the menu did not match their eating preferences and the processing method was less favorable. Also, some of them was having their eating preference based on their awareness of medical problem. Sometimes, older adults with underweight status was more likely to ask an additional portion, while overweight/obese older adults had their leftover food. Dietary patterns across generations have been shown to differ in previous studies (Sari et al., 2022). Puspa et al., (2019) states that the quantity of leftovers is much influenced by taste, appetite and the way of presentation (tidiness and cleanliness of cutlery).

Based on the observations, the most food leftovers were found on the third day. Although the average leftover food on the third day was just a few ($\leq 20\%$), it still needs to be noticed that almost half (42.9%) of the respondents have a lot of leftovers. Based on the type of food provided, the largest leftover food was at lunch with a menu of fried catfish and tamarind vegetable soup. Meanwhile, on other days, the most leftovers were found on the first day menu, namely fish sardines (pindang sarden) with 26.0% leftovers. The distribution of leftover food for 3 days menu can be seen at table 2 and table 3.

Beside food waste, consumer satisfaction with food service can also be an indicator of the success of a food service. The quality of food service can be assessed based on several indicators such as food quality (taste and appearance), punctuality, staff service, cleanliness of cutlery, menu variations, and the atmosphere of the dining environment (Nurqisthy et al., 2016; Puspa et al., 2019; Velawati et al., 2021). A better level of satisfaction is associated with a smaller decrease of nutritional status due to higher intake and less food waste (Semedi et al., 2013).

Food service satisfaction of the older adults at nursing home in Surabaya which is shown at table 4, most of the older adults are satisfied with the overall food service provided by nursing home in Surabaya (71.4%). At each indicator, food quality is the indicator that has the most dissatisfaction value (44.4%) compared to other indicators such as punctuality, staff service, cleanliness of cutlery, and the atmosphere of the dining environment.

Food quality, including the taste and appearance of food, contributes to increasing the attractiveness and appetite of consumers. Punctuality of food serving is related to the accuracy of the condition of hunger and satiety that affects one's appetite. Cleanliness of cutlery in fact can also affect someone's appetite. Dirty cutlery can make consumers feel disgusted and have no appetite. In addition, the dining environment and the staff service can affect the mood or psychology of consumers (Nawai et al., 2021; Semedi et al., 2013).

Residents' food satisfaction in this study shows a positive perspective. Most of older adults are satisfied and the most positive indicator is

Table 2. Food Waste on Each Type of Food at Griya Werdha Surabaya

Menu Cycle	Food Waste Mean (%)			Total
	Breakfast	Lunch	Dinner	
Day 1				
Staple food				
Rice	6.4	4.2	4.6	
Noodle soup	-	-	1.0	
Plant-based protein				
Tofu sardines	1.7	-	-	
Fried tofu	-	0.6	-	
Tofu (<i>perkedel</i>)	-	-	2.9	
Animal protein				
Fish sardines	26.0	-	-	
Vegetable				
Mix vegetables (<i>lodeh</i>)	-	11.4	-	
Snack/Fruit				
Banana/melon	-	2.0	-	
Steamed sweet potato	-	-	7.1	
Total				8.4±7.3
Day 2				
Staple food				
Rice	8,1	8,4	6,5	
Plant-based protein				
Tofu	9,3	-	-	
Tofu (<i>opor</i>)	-	-	7,5	
Animal protein				
Meatball	7,1	-	-	
Red soup sausage and chicken	-	18,9	-	
Shred chicken (<i>opor</i>)	-	-	7,1	
Vegetable				
Red soup	-	7,5	-	
Snack/Fruit				
Banana/orange	-	5,6	-	
Pudding	-	-	14,7	
Total				10,4±9,8
Day 3				
Staple food				
Rice	9,6	22,4	7,7	
Plant-based protein				
Fried tempeh	2,0	-	-	
Stew tofu	-	-	5,6	
Animal protein				
Fried catfish	-	45,8	-	
Stew meat	-	-	9,6	
Vegetable				
Stir carrot cauliflower	16,5	-	-	
Tamarind vegetable soup	-	32,0	-	
Snack/Fruit				
Banana/papaya	-	13,9	-	
Steamed sweet potato	-	-	17,9	
Total				17,4±15,9

Note: the use of ‘-’ means it was not on the menu at that meal time.

Table 3. Food Waste at Griya Werdha Surabaya

Day	Mean±SD (%)		Mean±SD (%)
	Few (≤20%)	Lots (>20%)	
1	56 (88.9)	7 (11,1)	8,4±7,3
2	54 (85.7)	9 (14.3)	10,4±9,8
3	36 (57.1)	27 (42.9)	17,4±15,9
Total			12.0±9.0

Table 4. Food Service Satisfaction by the Older Adults

Variable	Satisfied n (%)	Not satisfied n (%)
Food Service Satisfaction Indicators		
Food quality	35 (55.6)	28 (44.4)
Punctuality	55 (87.3)	8 (12.7)
Staff service	54 (85.7)	9 (14.3)
Cutlery cleanliness	49 (77.8)	14 (22.2)
Dining environment	51 (81)	12 (19)
Overall food service satisfaction	45 (71.4)	18 (26.8)

Table 5. Relationship between Food Waste and Food Service Satisfaction

Food Waste	Food Service Satisfaction		p value
	Not Satisfied n (%)	Satisfied n (%)	
Few	12 (19.0)	38 (60.3)	0.115
Lots	6 (9.5)	7 (11.1)	

food quality. This is in line with previous study that indicated indicators of food quality are the most influential aspects of patient satisfaction in general, while the indicator that is considered the most positive is the service of the staff (Messina et al., 2013). This is different from this study which shows that the indicator with the most positive value is the punctuality indicator.

The high satisfaction rating of the older adults at nursing home in Surabaya can be attributed to the different backgrounds of the older adults. Based on the results of interviews, most of the older adults are neglected or living-alone so that can affect the older adults by being more accepting of the situation and grateful for having a better life than outside the nursing home. In addition, most of the older adults have lived in the nursing home for 1-3 years so that they have more or less adapted well to the conditions in the nursing home.

Based on table 5, this study shows that there is no relationship between food waste and food service satisfaction ($p=0.115$). Basically, satisfaction can be presented in food waste (Simzari et al., 2017). Previous studies have stated that food service satisfaction is related to the level of consumer consumption (Divert et al., 2015; Heidi et al., 2017; Navarro et al., 2016). The higher the level of satisfaction, the less nutrients are lost, which means there is less leftover food. This study is not in line with the results found by Ronitawati et al. (Ronitawati et al., 2021) which shows that there was a relationship between the level of satisfaction with the value of missing nutrients ($p=0.0001$, $r=-0.34$).

The absence of this relationship might be caused by the good results of the older adults' food satisfaction that mostly positive. Length of stay was one of the institutional service satisfaction predictors. Residents with a longer length of stay had better life adjustments (Sun et al., 2020). Whilst for the food waste, in this study was more influenced by the residents' food preference and mostly high only in some specific menus.

Food service in nursing home is one factor that can affect the nutritional status of the older adults related to capability to provide nutritional needs of the older adults every day. Dissatisfaction with food service can increase the risk of malnutrition up to 20 times. The more satisfied the older adults with food service are, the better the nutritional status (Saghafi-Asl & Vaghef-Mehrabany, 2017). Nursing home in Surabaya allows the older adults to receive food from outside nursing home but they do not allow the older adults to buy food from outside. Frequency of relatives or family's visit can be considered quite rare, so most of the nutritional needs is provided by the nursing home. Therefore, the nursing homes must provide a proper menu that can fulfill the older adults' requirement.

The limitations of this study was the assessment of food service satisfaction is very subjective and influenced by the level of adaptation of the older adults to life in nursing home even though it has been carried out by direct interviews without the presence of the nursing home's staff. High food waste in this study tends to be more directed to the taste, preferences, and mood of the older adults. Besides that, short sample size in this

study was due to the inclusion criteria that only enroll independent' older adults which were only around 100 and must be excluded again with the other criteria. Further studies need to be done with a bigger scope area, not only in one nursing home, to avoid a biased result.

CONCLUSION

Food service satisfaction and food waste are important things to consider in the food service management system, especially for the older adults. There is no relationship between leftover food and food service satisfaction for the older adults at nursing home in Surabaya, this possibly due to the subjective answer of the older adults satisfaction in terms of their gratitude of being care in nursing home rather than living alone. Although the satisfaction score is good, there is a high amount of leftovers food on some of the menus served, so it is necessary to explore the acceptability of each menu served and older adults' preferences to minimize the amount of food waste. In addition, the menu cycle planning needs to be evaluated periodically and arrange by adjusting to the nutritional needs of the older adults.

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GUIDELINE FOR AUTHOR

I. GENERAL GUIDELINES

Papers submitted to the editorial are self-generated papers, scientific, contain contemporary issues and unpublished. To avoid duplication, the editor does not accept papers that are also sent to other journals at the same time for publication.

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III. WRITING SYSTEM

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Results and Discussion

Conclusion and Suggestion

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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

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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..

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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

Smith (2017) or (Smith, 2017)

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:

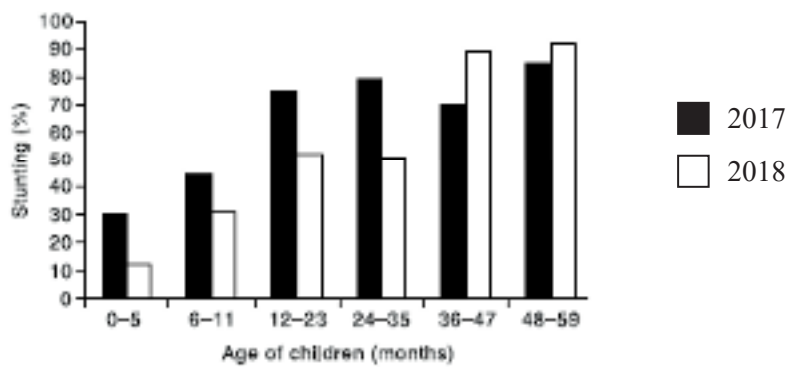


Figure 1. Changes in Stunting Prevalence (%) in Toddlers in Kalimantan

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