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THE IMPACT OF BISCUITS FORTIFIED USING PUMPKIN SEEDS ON NUTRITIONAL STATUS OF ADOLESCENTS: A RANDOMIZED SINGLE-BLIND CONTROLLED TRIAL

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ABSTRACT

This research assessed the effect of biscuit fortified using pumpkin seeds on nutritional intake, nutritional status, and plasma zinc level of the adolescent. As many as 40 participants were chosen randomly to be provided biscuit added by pumpkin seeds (intervention) and biscuit without additional pumpkin seeds (control) for 4 weeks. The study found that Intake for protein, fat, and energy increased significantly in the intervention group (p=0.012, p=0.004, p=0.003, respectively) but not in the control group. Similarly, micronutrient intakes, such as Fe and zinc, were also increased significantly (p= 0.000, p=0.003, respectively). There was no difference found in the nutritional status and zinc level between the intervention and control group after the intervention (2.7±10.28 vs 2.81±13.49, p>0.005). In conclusion, the consumption of biscuit added by pumpkin seeds can increase the nutritional intake so that it can be a healthy snack for the adolescent. A further trial measuring micronutrient level in the blood is warranted.

Key words: nutritious biscuit, pumpkin seed powder, nutrient intake, zinc

I. INTRODUCTION

Adolescent is a transition between childhood and adulthood. During this period, physical and psychological changes occur. WHO categorizes initial adolescent at the age of 10-14 years old, while final adolescent at the age of 15-19 years old Adolescent needs good nutrition to support growth and development [1]. Non optimal nutrition contributes to the inhibited and failed growth. Since adolescent is at fast growth period, then their nutritional intake must also be fulfilled adequately [2]. Investment on adolescents' nutrition has significant potential to increase the economy productivity, reproduction health and prevent chronic disease among the community [3].

In global, the prevalence of malnutrition on children and adolescent is 8.4% on female adolescent and 12.4% on male adolescent. The prevalence of obesity increased from less than 1% in 1975 to more than 5% on female adolescent and almost 8% on male adolescent in 2011. This prevalence does not experience decrease in three decades [4]. Nutritional issue gives negative effect on adolescent [5]. In addition to non-optimal growth, nutritional issue also affects the academic quality. Non-optimal nutritional status will also affect the learning ability of the adolescent [6].

Indonesian adolescents encounter nutritional problem [7]. One of the previous studies indicated that the Indonesian adolescents' intake has not fulfilled the daily Recommended Dietary Allowed (RDA). The lack of energy consumption reached 75.17% and the lack of fat consumption reached 74.82% [8]. Basic Health Research 2018 showed that the prevalence of thin adolescents at the age of 13-15 years old was 8.7% and obese adolescents was 16%. The prevalence of thin adolescents at the age of 16-18 years old was 8.1% and obese adolescents was 4.0%. In South Sulawesi, the prevalence of thin adolescents at the age of 13-15 years old was

10.8% and obese adolescents was 14.6%. Meanwhile, the prevalence of thin adolescents at the age of 16-18 years old was 10.4% and obese adolescents was 10.5% [9].

One of the important micronutrients needed by adolescents is zinc [3]. Zinc has important role in the growth and development of adolescents [10]. The lack of zinc will disturb the physical growth (stunting) and disturb the brain development [11] Unfortunately, most of zinc intake of the Indonesian adolescents has not fulfilled the nutritional Adequacy rate suggested [7]. Even though there was no study that has been conducted on measuring the prevalence of zinc deficiency on adolescents, but based on the zinc deficiency on children, it can describe the zinc deficiency possibilities on adolescents in Indonesia. In 2014, Nur Rahmah found that 54.8% of SDN Cambaya Ujung Tanah students experienced zinc deficiency [12]. Another study found that among 40 primary school students at the age of 9-12 years old in Semarang City involved as the research samples, all of them had zinc level below the standard [13]. In the same city, the other study was also done on 32 primary school students, finding that 75% of them experienced zinc deficiency [14]. New study was done to primary school students in Makassar City finding that 59.9% of them had low zinc level [15].

Various intervention strategies have been done to overcome nutritional problem on adolescents [16–19]. One of them is by performing fortification of food materials using other material which has good nutritional content. One of the food materials which has good nutritional contents is yellow pumpkin seeds [20]. A research that has been conducted obtained that in 100 gram of yellow pumpkin seeds, it contains 35.30% of protein, 36.30% of fat, 6.02% of carbohydrate, 14.20% of fibre, 0.10% of vitamin C, 6.5 mg of vitamin A, 573.03 ppm of calcium, 3.10 ppm of copper, 38 ppm of Fe 104, 68.87 ppm of zinc, 0.17% of phosphor, 0.33% of magnesium and 48 ppm of Manganese [21].

Adolescents often consume snack, so that nutritional snack making can become a strategy to overcome nutritional problem on adolescents. Biscuit is a type of snack preferred by people including adolescents. Therefore, biscuit can be developed into healthy snack by adding yellow pumpkin seeds flour which contains good nutrition. This research aimed to see the effect of yellow pumpkin seeds biscuit on the intake, nutritional status and zinc level of adolescents.

II. MATERIALS AND METHODS

Study Design and Sampling

Our study was a single-blind randomized controlled clinical trial (RCT) with 2 groups, conducted between July and November 2020. The protocol of the study was approved by The Ethics Committee of Universitas Hasanuddin of Public Health Faculty number 49201005007.

The number of research samples initially was 40 people, consisting of 20 people in the intervention group and 20 people in the control group. Based on the results of previous research and by choosing body weight as the main variable, the sample needed was 18 people per group with a significant level of 0.05. By considering the drop out of 10%, the number of samples for each group is 20 people. The participants who were involved to the end of the study were 30 people, because about 10 people were not present for blood collection during the endline. Participants who were not present because they moved due to the COVID-19 pandemic condition. The main outcomes measured in this study were macronutrient intake (carbohydrates, fat, protein and energy), micronutrient intake (Fe and zinc), body weight, height, and body mass index and plasma zinc levels.

Participants were obtained from an orphanage in Makassar City, because when the research was conducted, Makassar was carrying out the PSBB related to the Covid-19 pandemic. Therefore, the schools were carried out online leading to the research cannot be done at school. The inclusion criteria used were adolescents aged 10-18 years, had no chronic disease, and were willing to consume biscuits for 4 weeks. Adolescents who were on a diet program and consumed supplements or milk that contain high zinc were not involved in this study. The exclusion criteria used were the participants who experienced unpleasant effects during the study, violated protocols or the participant was no longer willing to continue to be participants in the study.

Intervention

The study protocol was explained to all study subjects. The participants then signed the informed consent as evidence of willingness. Participants were then randomized categorized into two groups, which are the group that received biscuits with added pumpkin seeds (intervention) and the group that received biscuits without additional pumpkin seeds (control). Biscuits were consumed for 4 weeks. The names of the participants were written on a piece of paper, then shuffled into a box. The names that came out were then grouped alternately into the intervention and control groups. The nutritional content of biscuits is shown in table 1.

The biscuits were made in the Culinary Laboratory of the Faculty of Public Health of Universitas Hasanuddin. The materials used were purchased from the local market. Each participant obtained 36 grams of biscuits per day. This amount was divided into 4 pieces weighing 9 grams. Participants were asked to consume 2 pieces of biscuits as a morning snack and 2 pieces as an afternoon snack. Participants did not know the contents of these biscuits because the biscuits were made in the same size, shape, taste and packaging but were labelled with the identification known only by the researchers. Since the research was conducted in an orphanage, the manager of the orphanage helped ensure that each participant consumed the biscuits. Biscuits should only be consumed with plain water, and not with tea or coffee. Researchers visited participants every week to bring 7 packages of biscuits in preparation for the next 7 days as well as evaluating the consumption of biscuits on the respondents. The composition of the biscuit per 100 grams was shown in Table 1.

Table 1. The Content of Control Biscuit and Yellow Pumpkin Seeds Biscuit

Variables	Wheat (100%)	Wheat: Pumpkin Seed (80:20)
Proximate Composition		
CHO (g)	6.4	17.33
Protein (g)	0.9	4.03
Fat (g)	10.7	11.89
Fibre (g)	0.29	0.59
Energy (kcal)	67.1	192.5
Micronutrient Composition		
Vit A (µg)	40.3	72
Vit C (mg)	0	2.969
Calcium (mg)	3.6	2.19
Potassium (mg)	9.7	13.23
Zinc (mg)	0.1	0.55

Outcome Measures

The anthropometric measurements included the measurements of body weight, height and body mass index that were performed at baseline and after 4 weeks of intervention (endline). The body weight was measured by the nearest 100-gram scale. During the measurement, the participants were asked to dress as little as possible. Height was measured using a microtois with a measurement to the nearest 0.1 cm. The body mass index was calculated by using the formula weight (kg) divided by height squared (meters).

Participants filled out a recall for 3 days, including 2 school days and 1 day off. The recall measurement was carried out before the study and when participants consumed the pumpkin seeds biscuits. Researchers used survey software to estimate the nutritional intake. The zinc level was measured by the Prodia laboratory, which is an accredited and standardized laboratory. Participants had 2 tubes of blood drawn, with 5 ml of each tube. Blood samples were stored in a cool box and immediately taken to the laboratory for further processing. Zinc levels were checked at baseline and endline. Zinc deficiency was defined as <75 µg/dl [22].

Statistical Analysis

Data analysis was performed using SPSS software (Version 22 for Windows). The normal distribution of the data was tested using Shapiro-Wilk one-sample test. Quantitative data are presented in the form of mean ± SD and the categorical data are presented as numbers and percentages. Data analysis in groups was performed using paired t-test l. In order to perform an analysis between the intervention and control groups, the free t test was used if the data were normally distributed, while Mc-Nemar was used if the data were not normally distributed. The significance value of $p < 0.05$.

III. RESULTS

The research flow diagram is shown in Figure 1. As many as 30 participants attended the research completely, while 10 people were not present at the time of collecting the last data.

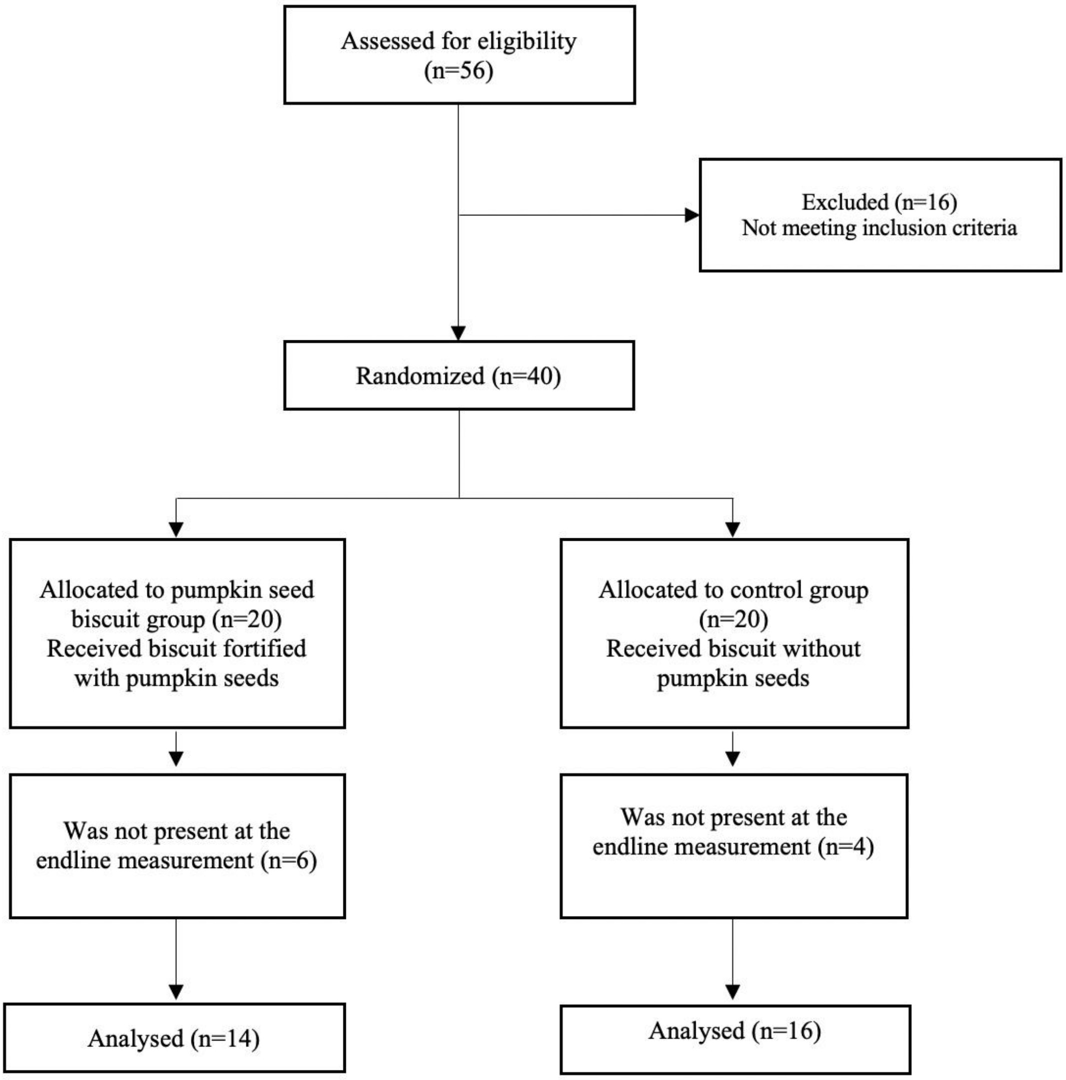

Figure 1. Research Flow Diagram

Table 2 shows the characteristics of the participants at baseline. According to the table, there were no significant differences in the characteristics of the participants.

Table 2. The Participants' Characteristics during the Baseline

Characteristics	Intervention n= 14	%	Control n= 16	%	p*
Gender					
Male	8	57.1	6	37.5	0.377
Female	6	42.9	10	62.5	
Age					
Initial adolescents	10	71.4	9	56.2	0.397
Final adolescents	4	28.6	7	43.8	
Educational Level					
Primary School	7	50	6	37.5	0.408

Junior High School	3	21.4	4	25	
Senior High School	4	28.2	6	37.6	
Nutritional Status					
Thin	6	42.9	10	62.5	0.429
Normal	7	50	4	25	
Overweight/obese	1	7.1	2	12.5	
Zinc Status					
Deficiency	4	28.6	4	25	0.886
Normal	10	71.4	12	75	

p* Mc Nemar

Table 3 shows the changes in the participants' nutritional intake. It appears that there was no difference between the nutritional intake in the intervention and control groups at the baseline and endline. However, in the intervention group, there was a significant increase in the intake of all types of nutrients ($p < 0.05$), except carbohydrates ($p > 0.05$), while in the control group, there was no significant difference in the intake between baseline and endline.

Table 3. Nutritional Intake on the Intervention and Control Groups after the Intervention

Nutritional Intake	Group								p*
	Intervention				Control				
	Mean	SD	min	max	Mean	SD	Min	max	
Macronutrient Intake									
Carbohydrate (gram)									
Pre	172.46	±80.01	106.93	291.8	149.28	±67.1	112.13	254.37	0,402
Post	201.62	±106.93	90,66	416.43	169.4	±82.78	127,63	320.53	0,370
p**	0.073				0.127				
Difference in mean	29.16±0.56				20.12±0.49				
Protein (gram)									
Pre	35.37	±14.42	18,7	63.73	35.66	±16.09	25.93	59.63	0,959
Post	45.84	±24.77	20	113.6	39.95	±19.18	26.93	74.37	0,478
p**	0.012				0.245				
Difference in mean	10.47±0.13				4.29±0.14				
Fat (gram)									
Pre	39.04	±19.07	12.73	81.37	38.15	±19.28	21.67	67.6	0,899
Post	56.24	±31.37	19.5	138.6	47.07	±25.41	26	90.27	0,391
p**	0.004				0.109				
Difference in mean	17.2±18.10				8.92±20.96				
Energy (Kcal)									
Pre	1170.91	±503.86	691.2	2132.2	1077.8	±477.01	816.84	1619.7	0,608
Post	1532.42	±732.44	740,53	3147.1	1273.9	±603.65	1042.5	2390.3	0,305
p**	0.003				0.067				
Difference in mean	361.50±362.21				196.13±397.21				
Micronutrient Intake									
Fe (mg)									
Pre	3.33	±1.91	1.9	7.07	3.6	±2.2	2.1	7.7	0,727
Post	8.6	±4.32	5.1	18.67	4.7	±2.7	2.73	10.33	0,008
p**	0.000				0.134				
Difference in mean	5.27±3.1				1.1±2.8				
Zinc (mg)									
Pre	3.4	±1.49	2.2	6.6	3.36	±1.57	2.73	5.77	0,907
Post	4.88	±2.64	1.8	11.97	4.2	±2.13	2.67	7.43	0,445
p**	0.003				0.071				
Difference in mean	1.45±1.52				0.84±1.71				

p* independent t-test; p** paired t-test

Table 4 shows the changes in nutritional status in the participants. There were no significant differences between the intervention and control groups, either at baseline or endline. However, when compared between baseline and endline in the intervention group, it appears to have a larger mean change. Body weight of participants in the intervention group increased by 0.17 kg (170 grams), while the control group increased by only 0.03 kg (30 grams).

Table 4. Nutritional status on intervention and control group during after and before the intervention

Nutritional Status	Group								p*
	Intervention				Control				
	Mean	SD	min	max	Mean	SD	min	max	
Weight (kg)									
Pre	40.21	±11.8	18.9	56.3	39.13	±14.48	22.7	70.5	0,823
Post	40.37	±11.62	19.4	57.2	39.15	±14.32	22.4	70.5	0,798
p**	0.590				0.857				
Difference in mean	0.17±1.16				0.03±0.68				
Height (cm)									
Pre	142.5	±13.03	116	161	144.43	±15.19	117.4	165.5	0,711
Post	142.54	±13.01	116	161	144.43	±15.19	117.4	165.5	0,724
p**	0.336				0.167				
Difference in mean	0.04±0.13				0.00±0.00				
BMI (kg/m²)									
Pre	19.36	±3.91	14.05	25.03	18.34	±5.08	14.3	33.53	0,540
Post	19.5	±3.95	14.41	26.11	18.41	±5.02	13.99	33.53	0,511
p**	0.424				0.458				
Difference in mean	0.14±0.62				0.06±0.34				

p* independent t-test; p** paired t-test

It also happened to the height. The intervention group experienced an increase in height by 0.04 cm (4 mm), while in the control group there was no increase in height. In terms of body mass index, the intervention groups increased by 0.14 kg/m2 while the control group increased by only 0.06 kg/m2.

Table 5 shows that there was no difference of the zinc levels between the intervention group and the control group. When compared between the baseline and endline, there was a decrease in plasma zinc levels in all groups. However, the decrease in the intervention group was lower than the control group. The intervention group experienced a decrease of 2.7 µg/dl, while the control group experienced a decrease of 2.81 µg/dl.

Table 5. Zinc Level on Intervention and Control Groups before and after the Intervention

Plasma Zinc Level	Groups								p*
	Intervention				Control				
	Mean	SD	min	max	Mean	SD	min	max	
Zinc Level (µg/dl)									
Pre	82.57	±10.51	66	108	84.03	±12.52	63	102	0,726
Post	79.86	±8.71	69	103	81.25	±15.54	58	123	0,761
p**	0,341				0,418				
Mean changes	2.7±10.28 (-)				2.81±13.49 (-)				

p* independent t-test; p** paired t-test

IV. DISCUSSION

The results of this research showed that the provision of biscuits fortified with pumpkin seeds can increase the nutritional intake. These results are in line with the previous research on the effectiveness of biscuits enriched with banana flour and tuna fish for underweight children. The study indicated an increase in energy and protein intake in the intervention group under five [23]. The results of this study are similar to the previous studies on the effects of the biscuits on children and adolescents who were hospitalized. The results showed that giving eel biscuits significantly increased the intake of energy, protein, fat, carbohydrates, zinc and vitamin A in the participants [24]. Several other studies have also shown that giving biscuits fortified with foods such as purple sweet potatoes [25], yellow sweet potatoes, tempeh and bran [26], tilapia fish [27], and snakehead fish [28, 29] can increase nutritional intake in the research participants.

In addition to increase the intake, the provision of biscuit can also be used to decrease the intake of obese sufferers. It is in line with the previous research on the provision of biscuit fortified using whey protein. The results indicated that the provision of the biscuit decreased the energy intake on the obese sufferers [30].

The increase of the participants' intake occurred due to the additional pumpkin seeds which can increase the nutritional content of the biscuit. The addition of yellow pumpkin seeds can increase the Fe and zinc content in the biscuit [31]. The addition of 33% pumpkin seeds flour on the biscuit can increase the Fe significantly compared to the control biscuit [20]. In addition, biscuits added with pumpkin seeds are also healthy biscuits that are rich in antioxidants and low on the glycaemic index [32]. Therefore, pumpkin seeds can be used as an alternative to overcome nutritional problems in children and adolescents [33].

The results of this research indicated that there was no change in body weight, height or body mass index in the participants, either between the intervention group and the control group or between the baseline and endline. However, there was a tendency for the intervention group to have better changes in all anthropometric parameters. The results of this study are similar to the previous research studying on the effect of giving cookies fortified with cowpea. Although there was an increase in body weight and BMI in the intervention group, the changes were not significant.

Such results are different from studies on the provision of Kawista biscuits to toddlers with malnutrition. The study showed that giving Kawista biscuits can significantly increase the participants' body weight [34]. Likewise, a study on the provision of eel biscuits for 3 months increased the participants' height significantly [24]. Another study found that giving biscuits for 1 year caused significant changes in BMI [35].

Body weight in adolescents is affected by many factors including nutritional knowledge and eating behaviour. In addition, several studies have found a significant relationship between physical activities, self-esteem and body weight in adolescents [36]. In addition, factors that affect body weight in adolescents are household income, peers and social networks [37], family habits [38], geography and access to food [39].

Height in adolescents is strongly related with stunting, even though the current study did not specifically assess the aspect of stunting. Adolescents need optimal height to prevent stunting. Particularly for adolescent girls, stunting becomes a risk factor for nutrition when these girls enter reproductive age. Stunting in young women is an intergenerational nutritional problem [40].

The explanation of why the changes in weight and height in this study were not significant, it can be due to the short intervention period of 4 weeks, although some studies have found changes in weight and height in the short intervention period. Body weight changes more noticeably, because body weight tends to change easily when compared to height. Meanwhile, the height itself is a parameter that takes longer to see the changes.

This study indicated that after providing the intervention, all participants experienced a decrease in plasma zinc levels. The results of this study are the same as research conducted on experimental animals, where after the intervention there was a decrease in plasma zinc levels in the intervention group and control [21]. The decrease in the intervention group could be due to the interaction between Fe and zinc [41]. As already explained, there was a significant increase in Fe intake in the intervention group. Fe levels that are too high can inhibit the zinc absorption. This is because they have similar physics-chemical properties such as atomic radius (iron 140 pm, zinc 135 pm) and oxidation (Fe2 +, 3 +, zinc2 +). In addition, both minerals have a major storage site in the liver and a maximum absorption site in the proximal duodenum. Although iron is only absorbed about 5% while zinc is 15%. Studies conducted in humans have shown an inhibitory effect of zinc on iron absorption when the two minerals are given together by fasting [42, 43].

The decrease of zinc levels in all groups could also be related to the participants' stressful condition. Since during the intervention the participants encountered a pandemic atmosphere which required them to learn from home, anxiety and depression can increase the body's need and use of zinc. Various animal and human studies have shown that zinc is related to the incidence of depression [44]. In mice, more aggressive and anxious behavior was shown in subjects with zinc deficiency when compared to controls [45, 46]. Experiments exposed to stressful situations, both chronic and acute, had significantly lower zinc concentrations than the control group.

In humans, low zinc levels are associated with mood disorders. This relationship seems consistent across all ages, from young adulthood [47] until old age [48]. Several studies have also shown a tentative relationship between zinc and mood regulation in infants and children [49]. Studies in Italy have shown an association between albumin concentration (an indicator of zinc status) and depression [50]. Studies conducted to assess zinc and Cu levels in depressed patients show that those with depression, both those with secondary anxiety and those who do not, have low zinc levels and high Cu levels [51].

In conclusion, biscuits with added pumpkin seeds can increase nutritional intake in adolescents. The increase in intake is due to the addition of pumpkin seeds to increase the nutritional content of biscuits, both macronutrient and micronutrient. Therefore, biscuits with added pumpkin seeds can be a healthy alternative snack for adolescents. This is in accordance with the situation of adolescents who like to eat snacks. It is expected that by consuming biscuits with added pumpkin seeds, it can meet the nutritional needs of the adolescents. So that adolescents can achieve optimal nutritional status and experience growth and development as expected.

Conflict of Interest

All authors declare accepting the manuscript and there is no conflict of interest

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Authors' contribution

All authors contributed equally in the study and in drafting the manuscript.

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