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ANALYSIS OF CALCIUM CONTENT, PHOSPHORUS AND TOTAL PLATE COUNT (TPC) CRACKERS FROM SNAKEHEAD FISH BONE (CHANNA STRIATA) AND KELOR LEAVES (MORINGA OLEIFERA) FLOUR

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ABSTRACT

Crackers are snacks usually only made from wheat flour with low micronutrients. Calcium and phosphorus minerals are needed, especially in adolescence to prevent osteopenia (early decline in bone mass). Snakehead fish bones and Moringa leaves are known to have high levels of calcium and phosphorus, so they can be substituted into crackers to create a healthy snack. This research aimed to determine the effect of substitution snakehead fish bone meal and Moringa leaves on calcium, phosphorus and Total Plate Count of crackers formulations in the selected and control formulations. This research was part of a large study where previous researchers have made formulations and organoleptic tests on crackers with 4 treatments and 3 replications and obtained the selected treatment in F1 with substitution of 5 g of snakehead fish bone flour and Moringa leaves as the most preferred formulation organoleptically. This research was conducted at Chem-Mix Pratama Yogyakarta. Laboratory data analysis used Independent sample t-test to test calcium and ALT levels, and Mann Whitney test to test phosphorus levels. There was a significant difference between the two formulas based on the treatment given. The nutritional content of selected crackers per 100 g include: calcium 377 mg/100 g, phosphorus 39.5 mg/100 g and Total Plate Count of 36 x 102 CFU/g was appropriate SNI crackers 2973-2011 which was <1x104 CFU/g. These crackers can be an innovative snack that is high in calcium for children and teenagers.

Keywords: Crackers; Snakehead Fish Bone Flour; Kelor Leaves Flour; Calcium, Phosphorus; TPC

Introduction

Nutritious intake is a necessity that has an important role in maintaining and improving health. In addition to considering nutritional and sensory factors, food selection must also pay attention to the function of disease prevention or as functional food.[1] Functional foods are natural foods that contain bioactive compounds in effective, non-toxic amounts and provide health benefits for the prevention, management, or treatment of chronic diseases or their symptoms.[2] Bioactive components in functional foods include amino acids, oligosaccharides, vitamins, minerals, sugar alcohols, dietary fiber, glycosides, isoprenoids, choline, prebiotics, polyunsaturated fatty acids and antioxidants.[3]

Low levels of calcium and phosphorus will lead to low mineralization of the matrix of new bone deposits. Phosphorus and calcium work together to form complex bonds that can help keep bones strong, so low levels of calcium and phosphorus can inhibit bone growth.[4] Peak bone mass

can be achieved by meeting calcium needs in children (7-9 years), who are nutritionally vulnerable groups and adolescents (10-15 years).

Factors that affect low bone mass include nutritional intake, especially calcium, phosphorus and low protein. Lifestyle in adolescents that occurs such as liking soft drinks, caffeinated (coffee and tea), fast food consumption and lack of physical activity can increase the risk of osteopenia which if not treated will lead to osteoporosis.[5] Early prevention age can be done by providing adequate calcium and phosphorus intake to children 7-9 years and adolescents from the age of 10-16 years, because at this age bone compaction and growth during adolescence take place quickly. By the time a child is 17 years old, 91% of the bone mass has formed, while the remaining 9% will form by the age of 20.

Investing in bones through the fulfillment of foods high in calcium and phosphorus from snacks and main foods as well as a healthy lifestyle, will help keep bones stronger and more resilient in old age.[6] One of the foods that can be used as a distraction to meet nutritional needs in the body, namely crackers with substitution of bone meal, snakehead fish and Moringa leaves is expected to add to the function of food for the growth of children and adolescents and as a prevention of disease, especially osteopenia or Early decrease in bone mass in adolescents with a high calcium and phosphorus content of crackers made.

In addition, the food processing process that is made, pays attention to the principles of food hygiene and sanitation, one of which is so that food is protected from microbial, physical, and chemical contamination.[7] To ensure products are safe for consumption for health, it is necessary to test safety on crackers products. In this study, the microbiological tests carried out were TPC (Total Plate Count) tests as quality aspects and food safety indicators indicated by the number of microbes in a product, contamination status and hygiene and sanitation at the time of production which is very important if the product is marketed in the future.[8] Based on SNI 2937:2011, the Total Plate Count of crackers meets the requirements if it shows a maximum number of 1x 104.[9]

Based on this background, an umbrella study was conducted to formulate snakehead fish bone meal and moringa leaves into crackers and researchers were interested in analyzing calcium and phosphorus levels to see if there was an effect of substitution of fish bone meal and moringa leaves in increasing calcium and phosphorus in control formulations and selected formulations and also testing TPC (Total Plate Count).

Methods

This study used an experimental design of laboratory analysis. This research was conducted in May-June 2022. This study is an umbrella study, as the first researcher will make a formulation and find the best formula of crackers and continue this research to analyze calcium, phosphorus levels and Total Plate Count of crackers products in the control formula and selected formula.

Calcium levels were analyzed using permanganometric methods, phosphorus with spectrophotometric methods and Total Plate Count were analyzed using ISO 4833 pouring method (Chem-Mix Pratama Yogyakarta Laboratory). This research has received approval from the Health Research Ethics Commission (KEPK) Sriwijaya University.

The calcium level test and Total Plate Number were carried out by analyzing the data using an independent sample t-test because the data is quantitative and has 2 unpaired treatments while the phosphorus level test is with the Mann-Whitney test. Tests of calcium, phosphorus levels and Total Plate Count conducted showed a significant difference in results (real) if the value of Sig.<0.05.

The ingredients used in this study were snakehead fish bone meal derived from waste taken from the 26 ilir market of Palembang, moringa leaf flour, low protein wheat flour, margarine, butter, water, skim milk, sugar, salt, yeast, water and baking powder. Materials for the analysis of crackers are solutions of HNO3, H2SO4, KMnO4, MR-BCG indicator, oxalic acid, ammonium meta vanadate, vanadat-molybdate solution, aquades, Agar Media Plate Count. Tools used in making crackers are digital scales, blenders, large tampas, basins, spoons, 80 mesh sieves, large pots, knives, stoves, baking sheets, ovens, ampia, brushes, and flower shape molds measuring 3 cm, diameter 3 cm for crackers. The tools used for analysis are Erlenmeyer, measuring pipette, measuring cup, measuring flask, Kjeldahl flask, kiln, filter paper, electric stove, porcelain crutches, vortex, spectrophotometer, Autoclave, Incubator, petri dish, digital scale, water bath spoon, plastic, spiritus, colony counting.

Fish bones are obtained by taking them at the 26 ilir market in Palembang which is not utilized. Fish bones and meat that are still attached are cleaned thoroughly and boiled using a steamer for 2 hours. Fish bones are cleaned again to remove any remaining meat and dirt that is still attached until clean. Ovening for 3 hours with a temperature of 60°C, after drying, the grinding process is carried out with a blender and sifted 80 mesh.

The next stage is the manufacture of crackers substituted with snakehead fish bone meal and moringa leaves. All ingredients including wheat flour, sugar, skim milk, baking powder, salt, water, yeast, margarine, butter, cork fish bone meal and moringa leaf meal are mixed evenly all in dough to produce a dough that is evenly mixed.[10] After that the dough is fermented for 30 minutes in a closed container. The dough is thinned using ampia with a thickness of 3 mm, then printed with flower prints measuring 3 cm, diameter 3cm, and ovening at 160 °C for 20 minutes. The formulation in making crackers for the substitution of snakehead fish bones and Moringa leaves consists of 4 different treatments, then selected formulations are obtained based on organoleptic tests to 25 panelists that have been carried out by the first researcher[11]. The formulation of crackers, snakehead fish bone meal and Moringa leaves can be seen in table 1

Table 1. Formulation of Crackers

Ingredients	Treatment			
	F0	**F1**	**F2**	**F3**
Low protein wheat flour	100 g	90 g	85 g	80 g
Cork fish bone meal				
Moringa leaf flour	0 g	5 g	7,5 g	10 g
Skim Milk	0 g	5 g	7,5 g	10 g
Margarine	4 g	4 g	4 g	4
Butter	20 g	20 g	20 g	20 g
Sugar	10 g	10 g	10 g	10 g
Salt	1 g	1 g	1 g	1 g
Baking Powder	2,2	2,2 g	2,2 g	2,2 g
Yeast	0,2 g	0,2 g	0,2 g	0,2 g
Water	2 g	2 g	2 g	2 g
Brown bars	34 mL	34 mL	34 mL	34 mL
Total Dough	25 g	25 g	25 g	25 g
Total Calcium	**198,4**	**198,4**	**198,4**	**198,4**
Total Phosphorus	**34 mg**	**206 mg**	**282 mg**	**334 mg**
	157 mg	**182 mg**	**194 mg**	**205 mg**

Results

Preliminary Research Physical Analysis of Snakehead Fish Bone Flour

The process of making snakehead fish bone meal consists of five stages including washing, steaming, drying in the oven, bone grinding with a blender, and sieving. The process of making snakehead fish bone meal is then carried out in the production kitchen of the Ministry of PUPR in the Karang Anyar area, Palembang.

Analysis of the physical properties of snakehead fish bone meal conducted in this study is the yield analysis of snakehead fish bone meal. Yield is the amount of fish bone meal produced from the ratio of the first weight of the material to the final weight of the material so that it is known how much weight of the material is lost during the process of making bone meal.[12] From 1 kg of fresh snakehead fish bones produce coarse snakehead fish bone meal of 552 g and 387 g of fine snakehead fish bone meal. The weight that decreases with the yield results is due to a decrease in water content, limited tools for smoothing bones that should use a flour grinder and there is flour flying during sieving because the particles of the sieve are very fine.

Analysis of Chemical Properties of Snakehead Fish Bone Flour

The results of the analysis of the physical chemical properties of snakehead fish bone meal conducted at the Chem-Mix Pratama Laboratory, Yogyakarta are presented in Table 2.

Tabel 2. Hasil Analysis of Chemical Properties of Snakehead Fish Bone Flour

Parameter	Snakehead Fish Bone Research	SNI**		
		Mutu I	Mutu II	Mutu III
Air (%)	7,75%	10	12	12
Abu (%)	60,34%	Max 20	Max 25	Max 30

**) SNI 01-2715-1996 Fishbone Flour

Table 2 shows that the moisture content yield of snakehead fish bone meal is 7.75%. Based on SNI 01-2715-1996 fish meal, this level has met the water content of SNI fish meal quality I which is a maximum of 10% and the content of fish bone meal according to SNI 1992-01-3158 which is a maximum of 8%. The ash content of snakehead fish bones produced is 60.34%. This ash content is classified as high exceeding the SNI 01-2715-1996 standard for quality III fish meal, which is a maximum of 30%.

Calcium Level Test Results

Analysis of the chemical properties of crackers was carried out at Chem-Mix Pratama Yogyakarta Laboratory. The chemical properties of selected crackers (F1) are compared to control crackers (F0). The chemical properties analyzed are calcium and phosphorus levels. The results of the analysis of the chemical properties of crackers are presented in Table 3.

Table 3. Results of Analysis of Calcium Crackers Levels in Selected Treatment (F1) and Control (F0)

Sample	Repeat		Average Calcium Level (%)	Average overall calcium content (%)
	1	2		
F0.1	0,8505	0,8991	0,8748	
F0.2	0,9790	0,9545	0,9667	0,929
F0.3	0,9229	0,9715	0,9467	
F1.1	3,7841	3,7594	3,7717	3,77
F2.2	3,6989	3,6493	3,6741	
F3.3	3,8433	3,8929	3,8681	

Source: Lab. Chem-Mix Pratama Yogyakarta

Table 3 found that calcium crackers substitution of snakehead fish bone meal and moringa leaves in the selected treatment (F1) which was carried out 3 times for each formulation found that the total calcium content in the selected formulation was 3.77% and in the control formulation the overall average calcium content was 0.929%. Based on the results of data analysis with the Independent sample t-test between control crackers and selected crackers, a significance score of 0.000<0.05 was obtained, there was a significant difference in average calcium levels between the control treatment group (F0) and the selected group treatment (F1).

Phosphorus Test Results

Table 4 found that phosphorus levels were carried out on crackers samples, substitution of snakehead fish bone meal and Moringa leaves carried out 3 times for each formulation with the average results obtained from each F0 and F1 treatment were 0.118% and 0.395%. Based on the results of the Mann-Whitney test between control crackers and selected crackers, a significance score of 0.002<0.005 was obtained. This means that H0 is rejected, so there is a significant difference in average calcium levels between the control treatment group (F0) and the selected treatment group (F1).

Table 4. Results of Analysis of Phosphorus Crackers Levels in the Best Treatment (F1) and Control (F0)

Sample	Repeat		Average Phosphorus Content (%)	Average overall phosphorus content (%)
	1	2		
F0.1	0,1156	0,1159	0,1157	
F0.2	0,1180	0,1176	0,1178	0,118
F0.3	0,1198	0,1194	0,1196	
F1.1	0,3926	0,3917	0,3921	0,395
F2.2	0,4014	0,4023	0,4018	
F3.3	0,3900	0,3891	0,3895	

Source: Lab. Chem-Mix Pratama

Total Crackers Plate Count Test Results

The microbiological analysis used is the analysis of the Total Plate Count (TPC) of food products. This analysis is carried out to see the number of colonies of microorganisms as a whole that grow in a food, so that the safety of a product can be known when consumed. The test results of ALT crackers can be seen in table 5.

Table 5. Results of Total Crackers Plate Count Analysis on Best Treatment (F1) and Control (F0)

Sample	Repeat		Rate-rate ALT (10^2) CFU/g	Average ALT (10^2) overall CFU/g
	1	2		
F0.1	32	37	34,5	
F0.2	47	44	45,5	41
F0.3	41	45	43	
F1.1	35	36	35,5	36
F2.2	32	34	33	
F3.3	39	42	40,5	

Source: Lab. Chem-Mix Pratama

Table 5 is known the results of Total Plate Count (TPC) analysis conducted on crackers samples carried out 3 times for each formulation with the average results of each F0 and F1 treatment are 41 x 102 CFU / g and 36 x 102 CFU / g. Based on the results of data analysis using the Independent sample t-test between control crackers and selected crackers, a significance score of 0.0028<0.005 was obtained, there was a significant difference in the average Total Plate Number (ALT).

Table 6 shows the contribution of nutritional fulfillment in crackers. From the results of macro substance content tests by the first researcher, it was found that crackers had fulfilled 40% of daily energy in children 7-9 years, 7.2% carbohydrates, 77.9% protein, and 175% fat.11 For calcium levels of 34% and phosphorus 3.4%. The interlude needs of crackers for teenagers meet 10% of the body's daily energy needs. The fulfillment of daily needs is said to be good in adolescents if the range is at 10-15% of needs or equivalent to 165-247.5 kcal. So, to meet the

nutritional content of interlude crackers that can be consumed is 38-76 g or 27-41 pieces of crackers per day.

Estimated Contribution of Selected Crackers Serving Rate

Table 6. Crackers' Contribution to the Fulfillment of Child Nutrition 7-9 years

Nutrients	Unit	Total nutrition/serving (38 g)	Suggested serving in a day (76 g)	%AKG of Adolescent Age Group 7-9 years
Energy*	Kkal	330,6	661,2	40
Carbohydrates*	Gram	Nutrients	18,24	7,2
Protein*	Gram	15,58	31,16	77,9%
Fat*	Gram	39,52	79,04	175%
Calcium	milligram	286,52	573,04	57,304
Phosphorus	miligram	30,02	60,04	12

Discussion

The results of the study found that snakehead fish bones used as flour for crackers formulations had a moisture content 7.75% lower than the SNI requirements for fish bone meal. Based on the Indonesian National Standard (SNI 01-3158-1992), fish bone meal has a maximum moisture content of 8%. Water content has an important role in a food because it can affect the quality and shelf life of food, so the lower the water content, the higher the shelf life. Low water content will make a food more durable if stored because in this condition the minimum number of microorganisms and insects.13 While the ash content of snakehead fish bone meal research is 60.34% higher than the SNI standard 01-2715-1996 quality III fish meal maximum 30%. This has to do with the mineral content of a foodstuff, the level of purity and the level of cleanliness of a foodstuff.

The results of calcium content analysis in the control formula crackers (F0) and selected (F1) in Table 3 found that there was an increase in calcium levels in the substitution of fish bone meal and Moringa leaves by 284.1 mg. This is in line with Fahreina14's research showing that crackers formulations added with Moringa leaf flour as much as 10 g, produce higher calcium levels of 324 mg / 100 g than control formulations of 188 mg / 100 g, as related to the content of Moringa flour which has calcium levels of 1324 mg / 100 g. Increased calcium levels with the addition of snakehead fish bone meal are also added to crackers. The control formulation on crackers without the addition of snakehead fish bone meal resulted in the lowest calcium content of 21.6 mg / 100 g.

While the addition of snakehead fish bone meal by 20 g from 40 g of wheat flour, resulting in calcium levels of 572.51 mg / 100 g.15 This is because, snakehead fish bone meal has high

calcium levels. The increase in calcium levels is also influenced by the addition of Moringa leaves compared to crackers that are only mixed with wheat flour in its manufacture. Moringa leaves contain higher calcium levels of 440 fresh leaves and 2003 mg if the leaves are dried.16 In making biscuits with the addition of Moringa leaf flour and tembang fish bones, it is known that calcium levels in the addition of 10% mixed flour (tembang fish bones and moringa leaves) from 200 g of wheat flour are 611 mg / 100 g greater than in the control treatment with calcium levels of 38 mg / 100 mg / 100 g. [17]

Phosphorus is the second most abundant mineral after calcium, which is as much as 1% of body weight. Phosphorus levels in bones and teeth are 80%, in blood and muscles about 10%, and 10% is widespread in chemical compounds.18 The results of phosphorus analysis in crackers based on Table 4 showed that there was an increase of 27.7 mg/100g between the control treatment group (F0) 11.8 mg/100 g and the selected treatment group (F1) 39.5 mg/100 g. This is in line with research conducted by Nuhalawang17 on making cookies with the addition of tembang fish bone meal and 30% Moringa leaves showed a higher phosphorus content of 19.06 mg / L compared to the control treatment of only 0.84 mg / L. The increase is because Moringa leaves have high phosphorus levels of 70 mg in fresh conditions and 204 mg if Moringa leaves are dried.16 While from snakehead fish bone meal in 100 g there are 549 mg / 100 g phosphorus. [12]

Total Plate Number (ALT) is a method for estimating the total number of microbes in a food. The method of calculating the number of microbes in the sample by counting the number of colonies of mesophilic aerobic microbes by pouring, dropping, and spreading.19 Total Plate Number that exceeds the limit, indicates the presence of disease-causing bacteria or pathogens such as Salmonella, E coli, shigella that can cause fever and diarrhea in humans.20 The results of Total Plate Number analysis on selected crackers (F1) and control (F0) are presented in Table 5. Based on the results of the Total Plate Number (ALT) analysis, it shows that in the control treatment (F0), the resulting Total Plate Number (ALT) value of 41 x 102 CFU/g is higher than the selected treatment (F1), which is 36 x 102 CFU/g. Based on the results of data analysis using the Independent sample t-test between control crackers and selected crackers, a significance score of $0.028<0.05$ was obtained, meaning that the treatment when added snakehead fish bone meal and Moringa leaves affected the Total Plate Number (ALT) of crackers. The lower total plate number in the selected formulation can also be because Moringa leaf flour has active compounds that are antimicrobial.

Another study conducted with the addition of Moringa leaf extract, made a decrease in total E. coli colonies. The antimicrobial effect contained in Moringa leaves has secondary metabolite compounds.21 Moringa leaf extract can also be used as an anti-bacterial (Pseudomonas Aeruginosa), a fish decay bacterium. In addition, other antibacterial compounds in Moringa leaves that are effective in damaging bacterial cell membranes such as saponins, triterpenoids, and

tannins.22 Water content is where microorganisms grow and multiply in number.23 The moisture content of crackers in control formulations is 5.615% higher than the selected formulation of 5.015%.11 This is in line with Kustiani 24's research in making crackers with the addition of moringa flour, can lower the moisture content of crackers.

The best snakehead fish bone meal crackers and Moringa leaves with a serving dose of 38 g for children aged 7-9 years have an energy content of 330.6 kcal, fat 39.52 g, protein 15.58 g, carbohydrates 9.12 g, calcium 286.52 mg and phosphorus 30.02 mg per serving.

By consuming 27-41 pieces of crackers or equivalent to 38-76 g for two interludes can meet energy needs by 40%, carbohydrate needs 7.2%, protein needs 77.9%, fat needs 175%, calcium needs 57.304% and phosphorus 12% a day in children (7-9 years). The calcium content in crackers is 207.35 mg / 100 g so that it can be claimed as a source of calcium because it has met the requirements for claims as a source of minerals, namely 15% ALG or 165 mg / 100 g per serving25. The phosphorus content in crackers, bone meal, snakehead fish and Moringa leaves, which is 30.02 mg / 100 g, cannot be claimed as a source of iron because it does not meet the nutritional claim requirements of 15% ALG or 60.04 mg / 100 g.

Conclusion

The addition of snakehead fish bone meal and moringa leaves to crackers has a significant effect on calcium levels, phosphorus, and Total Plate Number. Based on the results of organoleptic tests conducted by researcher 1, the selected formula is (F1) with the addition of snakehead fish bone meal and moringa leaves in the ratio of flour 90%: 10% mixed flour (snakehead fish bones and moringa leaves). The test results of selected formulation ALT crackers (F1) amounted to 36 x 102 CFU / g and met SNI crackers 2973-2011 which is <1x104 CFU / g. Further research needs to be done on the combination of ingredients to reduce the langu aroma in moringa in order to increase kinsmen's preference for crackers.

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Conflict of Interest

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