Check for updates

"TIS Biscuit" with High Content of Amino Acid and Mineral to Prevent Stunting

Erika Puspita(✉), Meilla Dwi Andrestian, and Siti Mas'odah

Nutrition and Dietetic, Polytechnic of Health Ministry of Health Banjarmasin, Banjarbaru, Indonesia
erikapuspita1836@gmail.com

Abstract. "TIS Biscuit" (cowpea tempe seluang fish) is a high protein and calcium snack which is important to prevent stunting. Stunting is a chronic malnutrition known as short toddlers. Increasing the stunted toddlers can be prevent according to adequate protein and calcium intake. This study aims to determine the levels of essential and nonessential amino acids, calcium, zinc, and phosphorus contained in TIS biscuits. This type of research is true experimental with a completely randomized design with two factors, namely cowpea tempeh flour (85%, 75%, 65%, and 55%) and seluang fish flour (15%, 25%, 35%, 45%). This research includes 3 stages, such as research stage I (organoleptic test of TIS), stage II (test of analytical hierarchy process), and stage III (comparison between control TIS (P0) and the selected biscuit). Statistical analysis of the results of organoleptic and chemical tests using the Independent Sample T-test. The selected biscuit which has the best organoleptic acceptance is TIS P1 (the proportion of 85% cowpea tempeh flour and 15% seluang fish flour). Amino acid, calcium, zinc, and phosphorus levels of TIS increased at P1. Statistical analysis showed significant differences between P0 and P1 on the color, smell, texture, flavour and almost all amino acid levels of TIS. On the other hand, there was no significant difference between P0 and P1 on calcium, zinc, and phosphorus levels. P1 can fulfill 100% of RDA for the lysine, calcium, and zinc by consuming 4 biscuits or 20 g per day as a snack.

Keywords: Biscuit · tempe · stunting

1 Introduction

Stunting means short or very short. Stunting is a toddler with chronic malnutrition. It is said to be short if the z-score value is less than -2SD and very short if the z-score value is less than -3SD based on length or height according to the age of the toddler [31]. Stunting increases the risk of illness, death, and the occurrence of motoric and mental growth disorders (Mitra, 2015). The prevalence of stunting among children is relatively high in South Kalimantan (33.1%), especially in Banjarbaru City at 39.73% [32].

Stunting is caused by inadequate intake of energy and nutrients as well as infectious diseases. Adequate and quality protein can support ideal bone growth and formation [53]. Foods containing protein are high quality depending on the composition and amount of

S. Februanti et al. (Eds.): MICon 2021, ASSEHR 708, pp. 912–932, 2023.
https://doi.org/10.2991/978-2-38476-022-0_102

essential amino acids [30]. Biscuits are a type of food that is practical, nutritious, has a variety of flavors, can be enjoyed at any time, and lasts a long time. Tempe as a local food source is high in protein from nuts, one of which is cowpea [24]. Cowpea is rich in amino acids, high quality, abundant supply, low price, and easy to obtain [1]. Cowpeas help relieve inflammation in the intestines which reduces the pain of stunting [14]. Cowpea tempeh has a preferred taste, protein digestibility, palatability, higher vitamin B levels (Fereirra et al., 2019). In addition to the protein content in tempeh, animal-based protein is also needed, called seluang fish which can improve the protein quality of biscuits [12]. Seluang fish (Rasbora spp.) are freshwater fish especially from Kalimantan which contains high protein and high calcium [19, 50]. Shurtleff and Aoyagi (2011) said that fresh tempeh and fish have a very short shelf life (48 h) and are very easily damaged by spoilage bacteria, so processing is needed into flour. Tempe flour and fish flour have a long shelf life. This drying process can increase usability, availability (Dewayani et al., 2020), add nutritional value, simplify handling, storage, minimize distribution costs, and easily mix with other ingredients [57].

Various studies have been conducted regarding the processing of tempeh and fish biscuit which have high protein values to prevent stunting. Research by Kurniadi et al. (2019) stated that the formulation of 63% MOCAF biscuits, 5% egg tempeh flour, 12% mung bean flour, and 20% catfish flour was the best for producing the highest sensory values and total chemical values that met the requirements of SNI 2011 and RDA 2013 [34]. The research of Salman et al. (2019) showed that the protein and fat value of biscuits can be increased by substituting 15% catfish flour, 15% soybean flour, and 70% wheat flour so that it includes functional biscuits as an alternative to preventing diabetes in toddlers [52]. This study was to evaluate the levels of essential and non-essential amino acids contained in seluang fish cowpea tempeh biscuits (TIS) as an alternative to preventing stunting. The results of this study are expected to add references to the formulation of local products made from cowpea and seluang fish to increase efforts to prevent stunting.

2 Materials and Methods

This research has been approved by the Research Ethics Commission of the University of Sari Mulia Banjarmasin. This type of research is experimental. The design of this research is True Experimental with the form of a Posttest-only Control Group Design. The study used a completely randomized design (CRD) with 5 (five) treatments and 5 repetitions (replication) obtained from the Federer formula. This study was conducted with 5 levels of treatment consisting of 1 control group and 4 levels of treatment with the formulation of cowpea tempeh flour (85%, 75%, 65%, and 55%) and seluang fish meal (15%, 25%, 35%)., and 45%), so the total sample size is 25 samples. The research materials were cowpea tempeh, seluang fish, and biscuits. The cowpea and seluang fish used were obtained from the Banjarbaru Market. Cowpea tempeh was made by researchers at the Microbiology Laboratory, Department of Nutrition, Health Polytechnic, Ministry of Health, Banjarmasin. The ingredients for making cowpea tempeh are cowpea and RAPRIMA instant yeast obtained from PT. Various Industrial Fermentation (AFI) Bandung. The ingredients for making TIS biscuits are wheat flour, refined sugar, cowpea tempeh flour, seluang fish flour, instant milk powder, chicken egg yolk, and margarine.

The tools in the study were divided into 3, namely the tools for making cowpea tempeh, flour-making tools, and biscuit-making tools. The tools for making cowpea tempeh include a basin, a steamer pan, a pan, plastic wrap, a toothpick, and a sieve. Tools for making flour, namely basin, steaming pan, oven, baking sheet, blender, knife, cutting board, aluminum foil, and a 60 mesh sieve. Biscuit-making tools, namely basin, mixer, spatula, cake mold, brush, and oven.

The research consisted of 3 stages, namely research phase I, phase II, and phase III. Phase I research is the organoleptic test of TIS biscuits. Phase I research was conducted for the first time to obtain organoleptic test results for all treatment TIS biscuits, except control TIS biscuits. The control TIS biscuit was compared with the most preferred TIS biscuit based on the Analytical Hierarchy Process (PHA) test. Phase II research is the Analytical Hierarchy Process (PHA) test. Phase II research aims to obtain the best treatment based on the organoleptic test results of TIS biscuits in phase I research. Phase III research aims to compare or analyze the differences between control TIS biscuits and TIS biscuits with the best treatment.

The process of making cowpea tempeh is done by fermentation. Cowpea tempeh flour and seluang fish flour are made by drying using a digital oven. TIS biscuits are made by baking in an oven. The organoleptic test was carried out using the Hedonic Scale Scoring method with the help of a questionnaire conducted on 30 moderately trained panelists. The analytical hierarchy process (PHA) test was carried out using the Expert Choice 2000 computer program. The levels of calcium, zinc, and phosphorus were tested at the Food Testing Laboratory of the Research and Industrial Standardization Institute (Baristand) Banjarbaru, while the levels of essential and non-essential amino acids were carried out at Saraswanti Indo Genetech (GIS) Bogor. Statistical analysis of the results of organoleptic and chemical tests was carried out with the Independent Sample T-test using the SPSS program with a significance of 0.05.

3 Result

Research Phase I

The organoleptic evaluation of TIS biscuits showed that the most preferred percentage of TIS biscuit color acceptance was P1 (76.7%) and the lowest value was P3 (56.7%) as shown in Table 1. Percentage of acceptance of biscuit smell. The most preferred TIS is P2 (70%) and the lowest value is P4 (56.7%). The percentage of acceptability of the most preferred TIS biscuit texture is P1 and P2 each (50%), and the lowest values are P3 and P4 each (43.3%). The percentage of acceptance of the TIS biscuit taste that was most preferred was P1 and P2 respectively (53.3%) and the lowest value was P3 (20%).

Research Phase II

The results of the analytical hierarchy process test (Table 2) show that the best treatment assessment for organoleptic parameters, namely TIS P1 biscuits (85% proportion of cowpea tempeh flour: 15% seluang fish flour).

Table 1. Percentage of Acceptance of TIS Biscuits in Research Phase I

Parameter	Hedonic Scale	P1	P2	P3	P4
Colour (%)	Poor	0	0	3,3	3,3
	Neither good nor poor	13,3	36,7	36,7	26,7
	Good	76,7	60	56,7	63,3
	Very good	10	3,3	3,3	6,7
Smell (%)	Poor	3,3	6,7	3,3	3,3
	Neither good nor poor	13,3	16,7	30	30
	Good	66,7	70	60	56,7
	Very good	16,7	6,7	6,7	10
Texture (%)	Poor	0	0	6,7	3,3
	Neither good nor poor	40	40	43,3	46,7
	Good	50	50	43,3	43,3
	Very good	10	10	6,7	6,7
Taste (%)	Poor	6,7	10	16,7	6,7
	Neither good nor poor	23,3	23,3	56,7	53,3
	Good	53,3	53,3	20	30
	Very good	16,7	13,3	6,7	10

Description: P0: 100% wheat flour; P1: 85% TK and 15% TI; P2: 75% TK and 25% TI; P3: 65% TK and 35% TI; P4: 55% TK and 45% TI; TK: cowpea tempeh flour; TI: seluang fish flour.

Table 2. The Best Treatment Decision Value in Research Phase II

TIS Biscuit Treatment	Decision Value
P1	0,361
P2	0,285
P3	0,170
P4	0,184

Description: P0: 100% wheat flour; P1: 85% TK and 15% TI; P2: 75% TK and 25% TI; P3: 65% TK and 35% TI; P4: 55% TK and 45% TI; TK: cowpea tempeh flour; TI: seluang fish flour.

Research Phase III

The results showed that the percentage comparison of the color acceptability of TIS biscuits showed different results. TIS biscuit color P0 (60%) was preferred over P1 (26.7%). The color produced by TIS P1 biscuits is almost close to P0 which is golden yellow (Fig. 1). The results of the Independent Sample T-test statistical test obtained a probability value of (0.000) $<$ (0.05), then Ho was rejected with the conclusion that

Table 3. Percentage of Acceptance of TIS Biscuits between Control and Best Treatment TIS Biscuits

Parameter	Hedonic Scale	P0	P1
Colour (%)	Poor	0	3,3
	Neither good nor poor	3,3	63,3
	Good	60	26,7
	Very good	36,7	6,7
Smell (%)	Poor	0	13,3
	Neither good nor poor	13,3	33,3
	Good	46,7	50
	Very good	40	3,3
Texture (%)	Poor	0	0
	Neither good nor poor	13,3	46,7
	Good	66,7	43,3
	Very good	20	10
Taste (%)	Poor	0	20
	Neither good nor poor	6,7	56,7
	Good	56,7	20
	Very good	36,7	3,3

Description: P0: 100% wheat flour; P1: 85% cowpea tempeh flour and 15% seluang fish flour.

there was a difference between the control TIS biscuit and the best treated TIS biscuit (P1) on the color acceptability of the TIS biscuit.

The smell of TIS biscuits P1 (50%) was more preferable to P0 (46.7%). The results of the Independent Sample T-test statistical test obtained a probability value of (0.000) < (0.05), then Ho was rejected with the conclusion that there was a difference between the control TIS biscuit and the best treated TIS biscuit (P1) on the acceptability of the TIS biscuit smell.

The texture of TIS P0 biscuits (66.7%) was more preferred than TIS P1 biscuits (43.3%). The results of the Independent Sample T-test statistical test obtained a probability value of (0.010) < (0.05), then Ho was rejected with the conclusion that there was a difference between the control TIS biscuit and the best treated TIS biscuit (P1) on the acceptability of the TIS biscuit texture.

The taste of TIS P0 biscuits (56.7%) was preferred over P1 (20%). The results of the Independent Sample T-test statistical test obtained a probability value of (0.000) < (0.05), then Ho was rejected with the conclusion that there was a difference between the control TIS biscuit and the best treatment TIS biscuit (P1) on the acceptability of the TIS biscuit taste. The percentage of acceptability of color, smell, texture, and taste can be seen in Table 3.

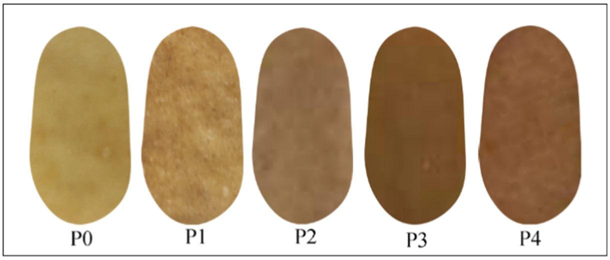

Fig. 1. TIS Biscuit Products

Evaluation of essential and non-essential amino acid levels in TIS biscuits P1 was higher than P0 (Table 4). The highest amino acid content in TIS P1 biscuits was glutamic acid, while the lowest was histidine. The results of the Independent Sample T-test statistical test obtained a probability value of less than 0.05 in almost all amino acids of TIS biscuits, except for the amino acid proline. The results of the study concluded that there were differences between control TIS biscuits and TIS biscuits with the best treatment (P1) on amino acid levels. Histidine, tyrosine, glycine, lysine, alanine, threonine, proline, isoleucine, valine, phenylalanine, arginine, serine, aspartic acid, leucine, and glutamic acid biscuit TIS ($p < 0.05$). But on the other hand, there was no difference in the proline content of TIS biscuits ($p > 0.05$).

The average calcium content of TIS biscuits P1 (6.79164 mg/100 g) was higher than P0 (4.7844 mg/100 g) as shown in Fig. 5.10. The results of the Independent Sample T-test statistical test obtained a probability value of 0.075 > (0.05), then H0 was accepted with the conclusion that there was no difference between the control TIS biscuits and the best treated TIS biscuits (P1) on calcium levels in TIS biscuits.

The average zinc content of TIS biscuits P1 (4.10614 mg/100 g) was higher than P0 (3.84344 mg/100 g). The average zinc content can be seen in Fig. 5.11. The results of the Independent Sample T-test statistical test obtained a probability value of 0.433 > (0.05), then H0 was accepted with the conclusion that there was no difference between the control TIS biscuits and the best treated TIS biscuits (P1) on the zinc content of the TIS biscuits.

The average phosphorus content of TIS biscuits P1 (0.1274%) was higher than P0 (0.1076%). The results of the Independent Sample T-test statistical test obtained a probability value of 0.559 > (0.05), then H0 was accepted with the conclusion that there was no difference between the control TIS biscuits and the best treated TIS biscuits (P1) on phosphorus levels of TIS biscuits.

TIS biscuits exhibit varying levels of amino acids, calcium, zinc, and phosphorus. The percentage of nutritional content fulfillment is calculated using the RDA. The percentage of RDA for several nutrients contained in 100 g of TIS biscuits can be seen in Table 5.

Table 4. Average Amino Acid and Mineral Levels between Control and Best Treatment TIS Biscuits

Amino Acid	P0	P1
Histidine*	290,2683	514,3458[a]
Tyrosine	391,494	538,0082[b]
Glysine	423,4597	747,2616[c]
Lysine*	350,8333	805,9984[d]
Alanine	398,0048	817,0428[e]
Threonine*	483,313	841,9462[f]
Proline	962,698	964,3008[g]
Isoleucine*	482,8832	972,2898[h]
Valine*	605,8649	1020,8126[i]
Phenylalanine*	619,6149	1043,8968[j]
Arginine	552,2183	1078,0462[k]
Serine	753,2876	1143,364[l]
Aspartic Acid	593,8041	1570,084[m]
Leucine*	874,4024	1541,236[n]
Glutamic Acid	2471,7206	3018,096[o]
Mikro Mineral		
Calcium	4,7844	6,79164
Zinc	3,84344	4,10614
Phosphor (%)	0,1076	0,1274

Description: * indicates essential amino acids. Lowercase letters in numbers indicate the order of the smallest to largest amino acid levels (a–o). P0: 100% wheat flour; P1: 85% cowpea tempeh flour and 15% seluang fish flour; The levels of amino acids, calcium, and zinc are displayed in units of mg/100 g.

4 Discussion

Research Phase I

Color

The most preferred color of TIS biscuits, which is light brown, is seen in TIS P1 biscuits. The light brown color of TIS P1 biscuits correlated with the results of the acceptability test, which showed a slightly sour taste because fermentation lowered the pH of the processed product. This is following the research of Andrestian, et al. (2010) which states that browning can be inhibited by the presence of acid formed from fermentation, resulting in a decrease in pH. In addition, the proportion of seluang fish flour that is small or large is thought to affect the natural dark brown color of TIS biscuits. The same thing was studied by Putri, et al. (2020) that the substitution of fish flour causes the color of the cookies to brown. Cookie formulations with the addition of 15% and 20%

Table 5. Percentage of Nutrient Adequacy Rate (RDA) of Several Nutrients Contained in 100 g TIS Biscuits

Age Group	Nutrients	RDA*	%RDA TIS Biscuit **	
			P0	P1
0 - 59 months	Lysine	75 mg***	467	1074
	Calcium	530 mg	0,90	1,28
	Zinc	3,025 mg	127	135
	Phosphor	333,75 mg	0,032	0,038

Note: * Source of 2019 RDA. ** % RDA = (nutritional content of biscuits × 100)/RDA of nutrients.
***Source Awobusuyi, et al. (2020).

seluang-banana flour showed a more brownish color of the cookies [50]. The brownish color of the biscuits is caused by the Maillard reaction during the baking process. This process causes amino acids to react with reducing sugars. TIS biscuits contain more amino acids than seluang fish flour. Therefore, the resulting brownish color is thought to be due to the substitution of seluang fish flour. This is following research that fish flour has a lower degree of whiteness than wheat flour, meaning that the more addition of fish flour the darker the color of the biscuits produced [38]. In addition, the same results show that the higher the substitution of fish flour, the resulting color tends to be darker [29]. TIS P3 biscuits are the least preferred because a certain proportion causes TIS P3 biscuits to be less preferred (56.7%). This is because the proportion of seluang fish flour in TIS P3 biscuits (35%) exceeds the color preference limit based on organoleptic tests. This is supported by Sari, et al. (2014) which shows that the preferred color of fish flour substitution biscuits is (10%–20%). This is thought to affect panelists' acceptance of color because certain proportions have been shown to reduce color acceptability. The higher the fish flour substitution, the lower the color characteristics of the processed product [41]. The color of seluang fish flour shows light brown to dark brown, while the color of good quality fish flour is light brown. The color of a good fish flour is light brown with a clean and bright appearance [6, 8]. The color percentage of TIS P1 biscuits was the most preferred (76.7%) with the proportion of 85% cowpea tempeh flour and 15% fish flour because it had a color that matched the criteria, namely light brown.

Smell

The percentage of acceptance of the favorite smell of TIS biscuits in all treatments ranged from (56.7%–70%). This happens because tempeh flour has a special characteristic smell, so the use of tempeh flour that is a lot or a little affects the smell produced. In addition, biscuits are processed with biscuit-making ingredients, such as margarine. The use of margarine and room butter in making cookies will make the cookies smell good [53]. The smell of TIS biscuits is also influenced by the tempeh flour used. This is because the proportion of cowpea tempeh flour (55%–85%) dominates and has a distinctive and sharp smell. The sharp and distinctive smell of tempeh flour is the result of the smell of mold mycelium mixed with the smell of free amino acids and the result of fat decomposition from the fermentation process [33].

The smell of TIS P2 biscuits was rated as the most preferred (70%). This is because the unpleasant smell of tempeh is covered by the smell of seluang fish. The unpleasant smell of tempeh flour is caused by the activity of the lipoxygenase enzyme which can hydrolyze polyunsaturated fatty acids and produce volatile compounds that cause unpleasant smells, namely ethyl phenyl ketone [35]. This research is in line with that conducted by Permatasari and Rahayuni (2013) that tempeh processed products with 25% fish substitution produce unpleasant smells that do not dominate. This was due to a decrease in the unpleasant smell of the peanuts with a mixture of fish which masked the unpleasant smell of the tempeh. The proportion of fish flour (25%) did not affect the smell of TIS biscuits. The results of the study and the opinions of other researchers showed that the smell of TIS P2 biscuits could be influenced by the proportion of cowpea tempeh flour that covered the fish smell so that the smell that emerged was unpleasant from the dominance of the formulated tempeh. This smell is acceptable because it does not cause a foul odor or unpleasant odor caused by certain components, such as ammonia contained in fish. A large proportion of tempeh flour with smaller fish flour combine to produce an acceptable smell (t does not show fishy). These results indicate that the proportion (75%) of cowpea tempeh flour and (25%) seluang fish flour can be the limit of the proportion that produces the panelists' favorite smell. The proportion of seluang fish flour that exceeds this limit (25%) can indicate a decrease in smell acceptance.

TIS P4 biscuit has the lowest level (56.7%) of preference for the smell. The smell of TIS P4 biscuits was considered the most fish smell compared to other treatments. The fishy smell of fish is caused by the compound TMA (trimethylamine) which gives fish its fishy characteristics. The formation of TMA occurs through several stages, first the oxidation of choline by bacteria that cut the trimethylammonium group from choline to form trimethylamine-oxide [25]. This is following the research of Siswanti, et al. (2017) which states that processed fish products have a more fishy or fishy smell compared to other samples. This is supported by Pratiwi (2013) which states that more use of fish flour makes the smell of fish in processed fish more pronounced. The higher the concentration of fish flour added to the biscuit formulation, the smaller the preference value for the smell. This is due to the strong smell of fish that affects the preferences of panelists who are not used to it so that the smell is considered low by the panelists. This is following the research of Vital, et al. (2018) that processed products are also new in value and have never been in the olfactory memory. The smell is assessed automatically by sensors that will elicit a response to identifying the smell of food in the subconscious when the panelists imagine the smell of food. When the panelists taste something, a smell sensor will appear which is similar to what they have felt before. The results show that the addition of food ingredients can affect the smell of biscuits [23].

The results of the study and several other researchers showed that the smell of TIS P4 biscuits was influenced by a volatile component in seluang fish, called trimethylamine which caused a fishy smell in TIS biscuits. In addition, the high proportion of seluang fish flour causes the fishy smell in TIS P4 biscuits to be more pronounced. This smell is considered new and affects the panelists' preference for the smell of TIS P4 biscuits, resulting in a decrease in the acceptability of smells in TIS P4 biscuits.

Texture

The four biscuit samples (P1, P2, P3, and P4) had almost the same texture organoleptically, which had a low level of hardness. This is due to the protein contained in substitute flour and low starch content. The substitution of cowpea tempeh flour and seluang fish flour causes no gluten content in the dough, so the dough is crumbly or has a low level of hardness. TIS biscuit hardness level can be influenced by biscuit formulation, protein content in flour, and starch content in flour. Cowpea tempeh flour and seluang fish flour do not contain gluten. The gluten content that is not present in fish causes the biscuit texture to soften [27]. The absence of gluten content causes biscuits to crumble easily because gluten plays a role in giving strength to the dough [45]. This is supported by Doporto, et al. (2017) that the firm texture is caused by the interaction of protein and starch by hydrogen bonding during dough development and baking. The texture of gluten-free biscuits is caused by gelatinization of starch and sugar at low temperatures and the development of a protein-starch structure is slower to occur (Doporto, et al., 2017).

Differences in the level of hardness and crunchiness are closely related to differences in the composition of the basic ingredients, especially the composition of amylose and amylopectin [10]. Starch is another component of flour that can affect the texture of biscuits. The starch content (amylose and amylopectin) of cowpea tempeh flour and seluang fish flour is much lower than wheat flour [37]. Low amylose content in the ingredients will be able to reduce the crispness of the resulting biscuits because the amylose in the ingredients will form hydrogen bonds with water in fewer quantities [10]. Therefore, during the oven process, the water will settle and leave a full space in the ingredients and make the biscuits crumble [10]. This study is following the research of Puspitasari, et al. (2017) which showed that gluten-free cookies produced a crunchy cookie that broke easily with a soft texture. The texture and crunch of the cookies are directly proportional because if the texture of the cookies produced is hard, then the crunchiness produced will be difficult to break [49]. Agustina's research, et al., (2017) showed that the variation of 15%, 20%, and 25% with a less crunchy texture was influenced by the addition of flour associated with the higher protein content in the flour formula used. Proteins without gluten will interact more strongly with swollen starch granules. The crunchiness of TIS biscuits is influenced by starch. The low starch value in TIS biscuits affected the biscuit structure to be not sturdy and the lower starch in each treatment resulted in biscuits that had poor crispness. This is possible because of the interaction of amylose and amylopectin components which play a role in the compactness of the biscuit texture [66].

The results of this study and other researchers showed that the texture of TIS biscuits had acceptable texture acceptability with a crumb texture characteristic (low hardness). This is due to the interaction of starch and protein components affecting the compactness of TIS biscuits. TIS biscuits have low starch and no gluten, which affects the texture of TIS biscuits to become crumbly.

Taste

The panelists' preference for the taste of TIS biscuits was the highest with the percentage (53.3%) found in TIS P1 and P2 biscuits. The panelists' preference for the taste of the lowest TIS biscuit with a percentage (20%) was found in the TIS P3 biscuit. An increase in panelists' preference for the taste of TIS biscuits occurred in TIS P4 biscuits with a percentage (30%). Panelists mostly liked TIS biscuits with the proportions of cowpea tempeh flour (75%–85%) and seluang fish flour (15%–25%) which produced a slightly sour taste in TIS biscuits. The greater the cowpea tempeh flour added to the TIS biscuits caused a slightly sour taste that covered the bitter taste caused by ammonia so that the taste of TIS P1 and P2 biscuits was slightly sour. The addition of a lot of seluang fish flour can cause a bitter taste caused by ammonia. Ammonia is abundant in fish, about 95% of ammonia is in the form of NH4+ in fish bodies [39]. Ammonia has an unpleasant taste so that the addition of large fish flour causes a bitter taste in TIS biscuits [44].

The slightly sour taste of TIS P1 biscuits could be caused by the high proportion of cowpea tempeh flour. A large amount of cowpea tempeh flour in P1 and P2 led the panelists to the typical taste of tempeh, which is sour due to fermentation. This is supported by the research of Sharma, et al., (2020) that cowpea tempeh causes a sour taste because during fermentation natural growth of Lactobacillus bacteria occurs at reasonable limits to produce lactic acid, so the pH of tempeh drops to between 4 to 5 [56]. Sulistiani and Hidayat (2020) reported that the production of tempeh produced 109 cfu/g lactic acid bacteria during fermentation. These lactic acid bacteria have a role in the acidification process of beans during soaking [60]. The use of high cowpea tempeh flour in TIS P1 and P2 biscuits caused a sour taste which was favored by most panelists so that TIS P1 and P2 biscuits were well received by the panelists.

The percentage of TIS biscuit taste acceptance indicates that TIS P3 biscuits are the least preferred because of their bitter taste. The bitter taste can be caused by the high proportion of seluang fish flour (35%). In addition, the formulation between 65% cowpea tempeh flour compared to 35% seluang fish flour was proven to produce a less favorable taste, so that the TIS P3 biscuit taste acceptance score had the lowest percentage (20%). Fitria's research, et al. (2018) showed that snakehead fish can improve the taste of processed products. The research differs from these results due to the use of the whole body of seluang fish down to the bone which can give a bitter taste after being swallowed. Fish has a certain taste depending on the active component of the taste in the fish and the variety of fish body parts used. Active components such as free amino acids, nucleotides and related compounds, organic acids, and inorganic ions present in the muscles and liver of fish produce a different taste, which is sweeter in muscle and bitter in the liver [55]. Processing of processed fish products with the whole body of the fish as in the TIS biscuit research produces a bitter taste. Some of the active components that can cause a bitter taste in TIS P3 biscuits are the amino acid arginine, peptides such as alanine-proline, glycine-phenylalanine, glycine-tyrosine, glutamate-phenylalanine, tyrosine-proline, phenylalanine-proline [55]. Glutamic acid is abundant in TIS biscuits which organoleptically produces a savory taste, but in processing TIS biscuits glutamic acid can combine with the amino acid phenylalanine, causing a bitter taste in TIS P3 biscuits. The bitter taste due to the amino acid phenylalanine combined with tyrosine should be able to increase the savory or umami taste below the concentration threshold in the presence of salt and free amino acids [55]. The cause of the bitter taste in TIS P3 biscuits is that the amino acid components combine and are at concentrations exceeding the threshold, causing a bitter taste that is less favorable.

The results of this study prove that a certain proportion of TIS biscuits increases the preferred taste. The increase in the acceptability of the TIS biscuit taste occurred at P4 which was influenced by the highest proportion of seluang fish flour. The taste of TIS P4 biscuit is savory with a fish flour component that dominates. The dominance of this proportion of seluang fish flour gives rise to a savory taste so that it becomes a driver of liking for TIS P4 biscuits according to research [13]. This is supported by Siswanti, et al., (2017) that the added seluang fish flour causes a savory taste that comes from protein nutrients which are hydrolyzed into glutamic acid, giving rise to an umami taste [59]. This study shows that there is a certain treatment, called the TIS P4 biscuit which has a savory taste so that certain panelists like it. The evaluation carried out by Bundle, et al. (2010) that taste is related to subjective assessment of age, adult panelists tend to prefer the taste to this treatment. The results showed that TIS P1 biscuits were considered to have the most favorable taste, but in fact, there was an increase in taste in TIS P4 biscuits, so that they could be further developed, especially for certain age groups (adults) [16].

Phase II Research

The results showed that TIS P1 biscuits were the main priority in selecting the best treatment based on the criteria of color, taste, smell, and texture with the highest weight value, namely 0.36 or 36%. The analytical hierarchy process test results in the best treatment with a hierarchical structure and uses various considerations to develop weights or priorities. The advantage of this method is that it combines the power of the sense of taste and logic from the results of the organoleptic test of the panelists, then synthesizes the judgments of color, taste, smell, and texture assessment into results that are following estimates intuitively presented on the considerations that have been made (Syah, 2014).

Phase III Research

Color

The light brown color of TIS P1 biscuits was caused by seluang fish flour. The proportion of seluang fish flour with a proportion of 15% is thought to affect the natural brown color of TIS P1 biscuits. The Indonesian National Standard indicates that the color of the biscuits produced must be normal. International standards indicate that fortifications must not cause unacceptable sensory problems (e.g. color, taste, smell, or texture) at the level of fortification intended, e.g. change to unpleasant, unpleasant, or unattractive [67]. The brown color of processed products with fortification such as fresh meat and fish undergoes a natural color change with a color assessment that does not deviate. Therefore, the light brown color can still be tolerated at the proportion of 15% fish flour. TIS P1 biscuits produced a dark color in all treatments and were significantly different from P0 which was influenced by the color of seluang fish flour. This is supported by Bukya, et al. (2013) which shows that chicken meat substitution biscuits produce darker colors influenced by chicken meat.

The results of the research and the opinions of several researchers above can be concluded that the light brown color is preferred because of the proportion of 85% cowpea tempeh flour and 15% seluang fish flour in TIS P1 biscuits produces an acceptable color. TIS P1 Biscuits contain several nutrients that are important for preventing stunting. The recommended consumption of TIS P1 biscuits is 4 biscuits or 20 g per day as a snack.

TIS P1 biscuits can meet 100% of the RDA (Nutritional Adequacy Ratio) for children's amino acids lysine, calcium, and zinc.

Smell

The smell of the TIS P1 biscuit that appears is the typical smell of cowpea tempeh fermentation and produces a more preferred smell. Evaluation of the smell of TIS biscuits P1 is preferable to P0 indicating the fact that the formulation of cowpea tempeh flour and seluang fish flour positively affects the smell of TIS biscuits. The smell that appears on the TIS P1 biscuit can come from the fermented cowpea tempeh flour. The volatile compounds produce a certain smell and contribute to the taste of the processed product. The smell in TIS P1 biscuits correlated with a slightly sour taste caused by cowpea tempeh fermentation and differentiated between TIS P1 and P0 biscuits. The high proportion of cowpea tempeh flour produces a distinctive fermented smell that masks the rancid or rotten smell due to ammonia in the fish's body. The smell sensor indicated that the TIS P1 biscuit did not have the rancid odor that is usually present in soybeans. The scent that m The appearance of TIS P1 biscuits was slightly sour which came from fermentation and this smell was acceptable to the panelists and was preferred over P0. The addition of fish flour can theoretically affect the smell of the biscuits. TIS P1 Biscuits have a distinctive fermented smell that dominates. This is caused by the evaporation of volatile compounds which increases during the fermentation process. In addition, the proportion of seluang fish flour (15%) has been prepared with a lime soaking process, to reduce the fishy smell. The presence of a new smell in TIS P1 biscuits is a combination of the right proportion of 85% cowpea tempeh flour and 15% seluang fish flour resulting in a distinctive fermented smell that is preferred over P0.

Texture

The TIS P1 biscuit has a crumbly texture, is less firm, looks rough, and has a large particle size. Different textures in TIS P0 biscuits, which are hard and have small particle sizes. TIS P1 biscuits were less preferred than P0 with different textures due to the characteristics of the flour, especially the protein and starch content of the substitute flour. The high level of hardness in the texture of TIS P0 biscuits is caused by the gluten content which gives hardness to the dough. Different things happened to TIS P1 biscuits which had a crumb texture due to the absence of gluten and low starch content in cowpea tempeh flour. The gluten content that is not present in fish is also the cause of changes in the texture of TIS P1 biscuits compared to P0 [27].

Taste

The taste of TIS P0 biscuits is sweet. The taste of TIS P1 biscuits is slightly sour. Biscuit taste can be affected by flour substitution. The slightly sour taste of TIS P1 biscuits could be caused by the high proportion of cowpea tempeh flour. A large amount of cowpea tempeh flour in P1 accompanied the panelists to the typical taste of tempeh, which is sour due to fermentation. Tempeh processed products have the characteristics of tempeh fermentation, causing a sour taste [56]. Another study revealed a distinctive taste obtained from the use of cowpea as the basic ingredient. A certain proportion when the sense of taste assesses the taste of processed peanut products, there is a certain taste [65]. The distinctive taste that appears in cowpeas is caused by the autoxidation of the fatty acids present in the nuts, resulting in several volatile compounds that give rise to a certain taste [65].

TIS biscuits do not show a bad taste, but there is a slightly sour and slightly savory taste that comes from monosodium glutamate. Typical sour taste sensation comes from sour organic taste, salty taste comes from sodium chloride, umami taste comes from monosodium glutamate, and sweet taste comes from sucrose solution [42]. The taste of TIS biscuits was assessed according to the results of chemical analysis, especially amino acids. The slightly savory taste in TIS P1 biscuits corresponds to the high content of glutamic acid and aspartic acid, thus giving a savory taste to TIS P1 biscuits. The bitter taste was considered not to appear in TIS P1 biscuits due to the sour taste and the dominant fermentation smell with the active components.

The taste that appears on the TIS P1 biscuit is influenced by the combination of the concentration of cowpea tempeh flour and seluang fish flour which results in taste acceptance and a slightly sour, slightly savory, and slightly sweet taste. The concentration or proportion of flour in TIS P1 biscuits resulted in an acceptable and tolerable taste. The taste in TIS P1 biscuits is indeed new when compared to P0, but the slightly sour taste is still acceptable and has other advantages, which are high in amino acids. The less sweet taste in TIS P1 biscuits can be added with other ingredients so that it is close to P0 which has a sweet taste.

Amino Acid Level

TIS P1 biscuits had a higher amino acid content due to the substitution of cowpea tempeh flour and seluang fish flour. TIS P1 biscuits contain high protein and amino acids as shown in Table 5. Some of the amino acids in TIS P1 biscuits contained certain low levels, namely the essential amino acid histidine and the highest, namely the non-essential amino acid glutamic acid. In addition to varying amino acid levels due to the high protein content of cowpea and seluang fish, cowpea's fermentation process produces cowpea tempeh with increasing amino acids. Tempeh fermentation causes an increase in free amino acids, which is associated with proteolysis caused by microbial enzymes in yeast and lactic acid bacteria (LAB) [18]. High-quality protein foods must have essential amino acids in a ratio proportional to human needs [43]. This is confirmed by comparing the amino acid content with the Amino Acid Adequacy Rate according to the FAO (Food and Agriculture Organization) for the age group (children 1–2 years) (Table 5). Overall, the results showed that TIS biscuits were a source of essential and non-essential amino acids and that fermentation significantly increased their nutritional value. The fermentation process is related to enzymes that hydrolyze proteins, thereby increasing some of the functional properties of TIS biscuits. Materials sourced from cowpea tempeh flour and seluang fish flour can be used for high protein food applications [17].

Calcium Level

The average calcium content of TIS biscuits did not differ significantly ($p > 0.05$) due to several baking processes that could remove calcium from TIS P1 biscuits. This is supported by Dwi and Maria (2017) who state that the calcium content decreases after the cooking process at temperatures above 100 °C. This research uses a biscuit baking temperature of 130 °C which exceeds the maximum limit of calcium breakdown temperature (100 °C). However, it is not known what percentage of calcium is lost due to the roasting process. This is because the researchers did not analyze calcium levels in cowpea tempeh flour and seluang fish flour and there is no information related to this in several references.

The average calcium content in TIS P1 biscuits was higher than P0. This is due to the use of all parts of seluang fish and tempeh. Seluang fish and cowpea contain 642 mg and 481 mg of calcium per 100 g, which are higher than 22 mg of wheat flour [20]. The results of the calcium level test in TIS P1 biscuits showed an increase due to the substitution of cowpea tempeh flour and seluang fish flour. The addition of fish flour (especially with the bones) can increase the calcium value of food products (biscuits) (Maheasy, 2013). The seluang fish flour formulated in TIS biscuits utilizes all parts of the fish, so that seluang fish flour can provide high levels of calcium.

Zinc Level

The average zinc content between TIS P0 and P1 biscuits was not significantly different ($p > 0.05$) or almost the same as the difference (2.627 mg/kg). The zinc content which was almost the same in TIS P1 and P0 biscuits was caused by the use of wheat flour in TIS P0 biscuits to increase zinc levels. Wheat flour is reported to contain high zinc at 30 ppm [11]. The high zinc content in TIS P1 biscuits was influenced by the substitution of cowpea tempeh flour. This is supported by Rahmi, et al. (2018) that tempehh contains minerals, namely zinc which remains high even though it has gone through the cooking process. The zinc content of the tempeh studied was relatively high, it was reported that the zinc content of raw and cooked tempeh did not differ much so that in the production of biscuits using the tempeh, the product automatically contained high zinc. This is following the research of Astuti, et al. (2014) which stated that there was no significant difference in zinc levels of raw and cooked tempeh. This is evidenced by the zinc content of raw and cooked tempeh (0.728 mg/100 g) and (0.542 mg/100 g) respectively [9]. Cowpea tempeh can be used as a source of high zinc material with increased bioavailability from (73%) to (84.5%) due to the fermentation process [64].

The formulation of TIS biscuits can affect the zinc content of TIS biscuits, although TIS P1 biscuits contain almost the same zinc as P0. The proportion of seluang fish flour in TIS P1 biscuits contributed to the zinc content. This is following the research of Pratiwi, et al. (2018) which states that the addition of fish flour increases the zinc content of biscuits because seluang fish contains 3.6 mg of zinc [20]. The substituted seluang fish flour adds a lot of zinc content to the biscuits even though seluang fish has been processed. This is because the mineral (zinc) is resistant to processing and during storage [9]. The increase in zinc content in TIS P1 biscuits occurred due to the substitution of cowpea tempeh flour and seluang fish flour which contained zinc in a resistant state during the processing, but high zinc content could also be obtained from wheat flour.

Phosphorus Level

The average phosphorus content of TIS biscuits did not differ significantly ($p > 0.05$) due to the baking process which removed phosphorus at P1. This is supported by Dwi and Maria (2017) who state that the cooking process exceeding 100 °C can reduce phosphorus levels significantly. This study uses a biscuit baking temperature of 130 °C which exceeds the maximum limit of the phosphorus breakdown temperature (100 °C). However, it is not known what percentage of phosphorus is lost due to the roasting process. This is because the researchers did not analyze phosphorus levels in cowpea tempeh flour and seluang fish flour and there is no information related to this in several references. The average phosphorus content in TIS P1 biscuits was higher than P0. This is caused by the substitution of seluang fish meal. The results of the study are almost the same as the research conducted by Agustini, et al. (2011) showed that the addition of simping flour to cookies resulted in an average phosphorus (0.37–0.53%). Sources of animal protein that are used to make biscuits usually contain high levels of phosphorus and this has been proven in this study.

The seluang fish used in the production of TIS biscuits is prepared whole with bones that are high in calcium. This can produce processed products that have balanced calcium and phosphorus compounds [61]. The phosphorus content of cowpea and seluang fish are 399 mg and 646 mg, respectively [20]. In addition, phosphorus content found in other food sources has been identified in TIS biscuits. Biscuit-making ingredients such as wheat flour and chicken eggs also contain 150 mg and 258 mg of phosphorus, respectively [20]. Phosphorus is found in almost all types of food, especially in red meat, fish, poultry, and wheat. Although other ingredients were also identified as containing phosphorus, the main source of phosphorus was food with high protein content, such as seluang fish and cowpea tempeh in TIS biscuits. Foods that are high in protein and calcium are usually also high in phosphorus [2]. Phosphorus is the second most abundant mineral component in the body after calcium, which is 1% of body weight. The role of phosphorus with calcium is to form the structure of bones and teeth [7]. The proportions of cowpea tempeh flour and seluang fish flour in TIS P1 biscuits resulted in products with higher calcium and phosphorus content than P0.

Percentage of Nutritional Adequacy Rate (RDA) in TIS Biscuits
Nutrients are needed by toddlers to prevent disease, support growth and development, and improve academic abilities. Snacks should be given to meet 10% of the child's daily needs for protein, energy, and some minerals and vitamins. In addition, it is also necessary to fulfill the nutritional requirements of the main food intake in a larger proportion and must be balanced according to balanced nutrition guidelines.

The percentage of nutritional adequacy rate (% RDA) given from 100 g of TIS biscuits produced for children is shown in Table 5. It can be seen that all the % RDA values for the amino acids lysine, calcium, zinc, and phosphorus were higher in TIS P1 biscuits than in TIS P1 biscuits with TIS P0 biscuits. Some are high in certain nutrients that produce 1 to more than 100% of the RDA, namely in the amino acid lysine (1074%), calcium (1.28%), and zinc (135.74%) per 100 g of TIS P1 biscuits with the Codex Alimentarius.

Fulfillment of amino acids lysine, calcium, zinc, and phosphorus per day can be done by consuming TIS P1 biscuits as many as 4 pieces of biscuits or 20 g as a snack that meets 100% of the RDA for the amino acids lysine, calcium, and zinc per day.

5 Conclusion

The selected treatment which had the best organoleptic acceptance was TIS P1 biscuit (the proportion of cowpea tempeh flour was 85% and seluang fish flour 15%). Percentage of acceptability of TIS P1 biscuit color (26.7%), taste (20%), smell (50%), and texture (43.3%). TIS P1 biscuits had significant differences in color, taste, smell ($p = 0.00$), and texture ($p = 0.01$). The selected TIS biscuits were P1 containing amino acids glutamic acid (30180.96 mg/kg), leucine (15412.36 mg/kg), aspartic acid (15700.84 mg/kg), serine (11433.64 mg/kg), arginine (10780.462 mg/kg), phenylalanine (10438.968 mg/kg), valine (10208.126 mg/kg), isoleucine (9722.898 mg/kg), threonine (8419.462 mg/kg), alanine (8170.428 mg/kg), lysine (8059.984 mg/kg), glycine (7472.616 mg/kg), tyrosine (5380.082 mg/kg), and histidine (5143.458 mg/kg) which were significantly different ($p < 0.05$), except for the amino acid proline ($p = 0.940$). TIS P1 biscuits contain an average of calcium (67.91 mg/kg), zinc (41.06 mg/kg), and phosphorus (0.12%). TIS P1 biscuits were not significantly different to calcium ($p = 0.068$), zinc ($p = 0.760$), and phosphorus ($p = 0.511$). TIS P1 biscuits can be given as much as 4 biscuits or 20 g per day as a snack to meet 10% of the amino acid needs of lysine, calcium and zinc in children.

Acknowledgments. The author would like to thank RISTEK-BRIN in the 2020 Indonesian Innovation Talent Strengthening Program has provided financial support for this research.

References

1. Adino, S., Wondifraw, Z. & Poultry, M. A., 2018. Replacement of Soybean Grain with Cowpea Grain (Vigna unguiculata) as Protein Supplement in Sasso x Rir Crossbred Chicks Diet. Fisheries & Wildlife Sciences, 6(1).
2. Afrinis, N., Verawati, B. & Harahap, D., 2018. Formulation and Characteristics of Vermicelli High in Protein and Calcium with Addition of Catfish Bone Flour (Pangasius Hypopthalmus) for Stunting Toddlers. Indonesian Public Health Media, 14(2).
3. Agustina, F., Susilo, J. & Wayansari, L., 2017. The effect of variations in the mixing of mung bean flour on the manufacture of "casein-free gluten-free" biscuits with mocaf flour as raw material on chemical characteristics and acceptability, Yogyakarta: Health Polytechnic of Yogyakarta Ministry of Health.
4. Agustini, T. W., Ratnawati, S. E., Wibowo, B. A. & Hutabarat, J., 2011. Utilization of scallop shells (Amusium pleuronectes) as a source of calcium in extrudate products. Indonesian Journal of Fishery Products Processing , 14(2), pp. 134-142.
5. Andrestian, M. D., Dewi, Z. & Novita, S., 2010. The amino acid content in mandai cempedak is the result of traditional and controlled fermentation. Banjarbaru, Banjarmasin Health Polytechnic.
6. Anggriani, A. N., Pujaningsih, R. I. & Sumarsih, S., 2019. Effect of Different Processing Methods and Levels of Giving Kersen Leaf Extract (Muntingia calabura L.) on Organoleptic Quality of Rucah Fish Meal. Indonesian Journal of Animal Science , 14(3), pp. 282–291.
7. Asikin, A. N., Kusumaningrum, I. & Hidayat, T., 2019. Effect of knife-fish bone powder addition on characteristics of starch and seaweed kerupuk as calcium and crude fiber sources. Current Research in Nutrition and Food Science.
8. Assadad, L., R., H. A. & N., W. T., 2015. Quality of trash fish meal in various processing processes. Yogyakarta, National Seminar on Fisheries and Marine Research Results.
9. Astuti, R., Aminah, S. & Syamsianah, A., 2014. The nutritional composition of tempeh fortified with iron and vitamin A in raw and cooked tempeh. Agritech, 34(2), pp. 151-159.
10. Ayu, M. S., Astuti, N., Nurlaela, L. & Kristiastuti, D., 2021. Effect of Substitution of Broccoli (Brassica Oleracea L. var italica) Powder on Organoleptic Properties of Cat's Tongue Cake. Journal of Cuisine, 10(2), pp. 267–276 .

11. National Standardization Agency, 2009. Wheat flour as food ingredient, s.p.: Indonesian National Standard.
12. Berrazaga, I., Micard, V., Gueugneau, M. & Walrand, S., 2019. The Role of the Anabolic Properties of Plant- versus Animal-Based Protein Sources in Supporting Muscle Mass Maintenance: A Critical Review. Nutrients, 11(8), p. 1825.
13. Biro, B., Maria A. S., Aniko Kovacs, Katalin Badak-Kerti, Klara Pasztor-Huszar, Attila Gere, 2020. Cricket-Enriched Oat Biscuit: Technological Analysis and Sensory Evaluation. Foods, 9(1561), pp. 1-17.
14. Budge, S., Parker, A. H., Hutchings, P. T. & Garbutt, C., 2019. Environmental enteric dysfunction and child stunting. Nutrition Reviews, p. 240–253.
15. Bukya, A., Sunooj, K. V. & Babu, A. S., 2013. Standardization and Evaluation of Physical, Textural and Organoleptic Properties of Chicken Biscuits. International Journal of Advanced Research, 1(6), pp. 163-168.
16. Bundel, M. C., Osundahunsi, O. & Akinoso, R., 2010. Supplementation of biscuit using rice bran and soybean flour. African Journal of Food Agriculture Nutrition and Development, 10(9).
17. Chan, Y.-J. Wen-Chien Lu, Hui-Yao Lin, Zong-Ru Wu, Chen-Wei Liou, Po-Hsien Li, 2020. Effect of Rice Protein Hydrolysates as an Egg Replacement on the Physicochemical Properties of Flaky Egg Rolls. Foods, 9(245), pp. 1–12.
18. Dewi, I. W. R., Anam, C. & Widowati, E., 2014. Sensory characteristics, nutritional value and antioxidant activity of gude (Cajanus cajan) and cowpea (Vigna unguiculata) tempeh with various variations in fermentation time. Biopharmaceutical, 12(2), pp. 73-82.
19. Directorate of Community Nutrition, 2017. Guidebook for Community Based Nutrition Recording and Reporting (e-PPGBM) Offline. Jakarta: Ministry of Health.
20. Directorate General of Public Health, 2018. Table of Indonesian Food Composition 2017. Jakarta: Ministry of Health of the Republic of Indonesia.
21. Doporto, M. C., Sacco, F., Viña, S. Z. & García, M. A., 2017. Quality and Technological Properties of Gluten-Free Biscuits Made with Pachyrhizus ahipa Flour as a Novel Ingredient. Food and Nutrition Sciences, Volume 8, pp. 70-83.
22. Dwi, A. A. & Maria, I., 2017. Substitution effect of wheat flour with moringa leaf toward the nutrient improvement and the quality of moringa biscuit (moringga oleifera). International Journal of ChemTech Research, 10(9), pp. 949-956.
23. Ernisti, W., Riyadi, S. & Jaya, F. M., 2018. Characteristics Of Crackers (Biskuit) Which Fortified With Catfish (Pangasius hypophthalmus) Fish Flour at Different Concentration. Jurnal Ilmu-Ilmu Perikanan dan Budidaya Perairan, 13(2).
24. Fadillah, A. Meillina F., Novi N., Syura A. A. A.W., Decy E., 2009. Development of Jackfruit Derivative Products through the Utilization of Jackfruit Seeds as Raw Material for Healthy Varonyil (Variation of Unyil Bread). IPB Repository.
25. Faisal, Patadjai, A. B. & Sadimantara, M. S., 2020. Chemical Study of Milkfish (Chanos chanos Forskal) Boneless Using Different Packaging Methods in Cold Temperature Storage (5°). J. Fish Protech , 3(2).
26. Fitria, E., Prabowo, S., Yuliani & Muflihah, 2018. Effect of Different Processing Methods on Chemical Composition and Sensory Characteristics of Processed Haruan Fish (Channa striata). Jurnal Teknologi Pertanian Universitas Mulawarman, 13(2), pp. 39-44.
27. Fitri, N. & Purwani, E., 2017. Effect of Flour substitution on pufferfish (Rastrelliger brachysoma) on protein content and biscuit acceptance. National Seminar on Nutrition 2017 UMS Nutrition Science Study Program "Optimization Strategy for Child Development", pp. 139–152.

28. Gomez, C., 2004. COWPEA: Post-Harvest. Dalam: M. D., penyunt. Rome, Italy: Operations Food and Agriculture Organization of the United Nations (FAO).
29. Hamidah, N., Mia Fransisca & Indah Asrifah, 2017. Effect of Substitution of Pumpkin Flour, Dumbo Catfish Flour on Nutritional Value, Biscuit Organoleptic Quality. 7(3).
30. Hardinsyah, Riyadi, H. & Napitupulu, V., 2013. Adequacy of Energy, Protein, Fat and Carbohydrates.Research Gate, pp. 1–26.
31. Ministry of Health Indonesia, 2016. Short Toddler Situation, Jakarta: Ministry of Health RI Data and Information Center.
32. Ministry of Health Indonesia, 2019. South Kalimantan Provincial Report Basic Health Research 2018, Jakarta: Community Research and Development Agency.
33. Khrisanapant, P., Kebede, B., Leong, S. Y. & Oey, I., 2019. A Comprehensive Characterisation of Volatile and Fatty Acid Profiles of Legume Seeds. Foods, 8(12), p. 651.
34. Kurniadi, M. Mukhamad Angwar, Miftahkussolikhah, Dian Rachmawanti Affandi, Nila Khusnia, 2019. Characteristics of Cookies From a Mixture of Modified Cassava Flour (MOCAF), Tempe, Eggs, Green Beans and Catfish. Journal of Industrial Research Dynamics, 30(1), pp. 1–9.
35. Kurniawati, K. & Ayustaningwarno, F., 2012. Effect of Wheat Flour Substitution with Tempe Flour and Yellow Sweet Potato Flour on Protein Content, -Carotene Content, and Organoleptic Quality of Sweet Bread. Journal of Nutrition College, 1(1), pp. 344-351.
36. Mahmudah, S., 2013. Effect of substitution of catfish bone meal (Clarias batrachus) on calcium content, hardness, and acceptability of biscuits, Surakarta: University of Muhammadiyah Surakarta.
37. Mamat, H. & Hill, S. E., 2018. Structural and functional properties of major ingredients of biscuit. International Food Research Journal , 25(2), pp. 462-471.
38. Mervina, C. M. K. & Marliyati, S. A., 2012. Biscuit Formulation With Substitution of Dumbo Catfish Flour (Clarias Gariepinus) And Soy Protein Isolate (Glycine Max) As Potential Foods For Undernourished Toddlers. Journal of Food Technology and Industry, 23(1).
39. Miramontes, E.Paul Mozdziak. James N. Petitte. Magdalena Kulus., Maria Wieczorkiewicz. Bartosz Kempisty, 2020. Skeletal Muscle and the Effects of Ammonia Toxicity in Fish, Mammalian, and Avian Species: A Comparative Review Based on Molecular Research. International Journal of Molecular Science, 21(4641), pp. 1–14.
40. Mitra, M., 2015. The Problem of Stunting and Interventions to Prevent Stunting (A Literature Review). Community Health Journal, Volume 2, p. 254.
41. Mohamed, G., Sulieman, A., Soliman, N. & Bassiuny, S., 2014. Fortification of Biscuits with Fish Protein Concentrate. World Journal of Dairy & Food Sciences , 9(2), pp. 242-249.
42. Ng'ong'ola-Manani, T. A. Tinna. Agnes M Mwangwela. Reidar B Sch€uller. Hilde M, Østlie. Trude Wicklund, 2014. Sensory evaluation and consumer acceptance of naturally and lactic acid bacteria-fermented pastes of soybeans and soybean–maize blends. Food Science & Nutrition, 2(2), pp. 114–131.
43. Omoba, O. S. & Isah, L. R., 2018. Influence of Sourdough Fermentation on Amino Acids Composition, Phenolic Profile, and Antioxidant Properties of Sorghum Biscuits. Prev. Nutr. Food Sci. , 23(3), pp. 220-227.
44. Pambudi, S., 2015. Effect of Proportion of Baking Soda (Sodium Bicarbonate) and Powdered Ammonia (Ammonium Bicarbonate) as Developers on Physical, Chemical and Organoleptic Properties of Bagiak Cake, Malang: Universitas Brawijaya.
45. Patimah, S., Arundhana, A. I., Mursaha, A. & Syam, A., 2019. Development of Foxtail Millet and Flying Fish Flour-Based Cookies as Functional Food. Current Research in Nutrition and Food Science , 7(2), pp. 504-516.
46. Permatasari, P. K. & Rahayuni, A., 2013. Tempeh nuggets with tilapia fish substitution as an alternative source of protein, fiber, and low fat. Journal of Nutrition College, 2(1).

47. Pratiwi, F., 2013. Utilization of Fly Fish Meat Flour for Making Fish Sticks, Semarang: Semarang State University.
48. Pratiwi, N. I. A. D., Nadimin & B, T. D. K., 2018. Substitution of skipjack viscera flour in biscuit making to increase nutrition. Food Nutrition Media, 25(2), pp. 44–49.
49. Puspitasari, V. A., Angkasa, D. & Wijaya, H., 2017. Making Gluten Free Cookies Made from Mocaf Flour and Cracked Rice Flour with Dates Extract Added, West Jakarta: Esa Unggul University.
50. Putri, A. S., Kusfriyadi, M. K. & Sera, A. C., 2020. Effect of Selpis Flour Substitution (Seluang and Banana) on Protein, Calcium, Acceptability and Organoleptic Quality of Cookies. Nutrition Research Journal, 8(1), pp. 25-31.
51. Rahmi, S. L., Mursyid, M. & Wulansari, D., 2018. The formulation of spiced tempeh and nutritional content testing. Industria: Journal of Agroindustrial Technology and Management, 1(7), pp. 57–65.
52. Salman, Y., Khadijah, S. & Suryani, N., 2019. Analysis of Macro Nutrient Contents Biscuits With Catfish Flour And Soybean Flour Formulations In An Effort To Prevent Stunting. Indonesian Health Journal, 10(1), pp. 17-22.
53. Sari, D. F., 2016. Differences in the Use of Margarine on Sensory Quality, Preference and Nutritional Content of Purple Sweet Potato Biscuits (Ipomoea Batatas var ayamurasaki), Semarang: Faculty of Engineering, State University of Semarang.
54. Sari, D. K. Sri Anna M., Lilik K., Ali K., Tommy Marcelino G, 2014. Organoleptic test of functional biscuit formulation based on snakehead fish meal (Ophiocephalus striatus). Agritech, 34(2).
55. Sarower, M. G., Hasanuzzaman, A. F. M., Biswas, B. & Abe, H., 2012. Taste producing components in fish and fisheries products: A review. Intl. J. of Food. Ferment. Technol, 2(2), pp. 113-121.
56. Sharma, R. Prakrati G., Pradeep K., Shashi Kant B., Saurabh K., 2020. Microbial Fermentation and Its Role in Quality Improvement of Fermented Foods. Fermentation, 6(106), pp. 1-20.
57. Shaviklo, G. R. Gudjon T., Sigurjon A., Kolbrun S., Hordur K., 2010. The influence of additives and drying methods on quality attributes of fish protein powder made from saithe (Pollachius virens). Journal of the Science of Food and Agriculture, pp. 2133–43.
58. Shurtleff, W. & Aoyagi, A., 2011. History of Tempeh and Tempeh Products (1815–2011): Extensively Annotated Bibliography and Sourcebook. ISBN: 9781928914396 penyunt. Lafayette, Louisiana: Soyinfo Center.
59. Siswanti, S., Agnesia, P. Y. & A, R. B. K., 2017. Utilization of mackerel meat and bones (Rastrelliger kanagurta) in making snack sticks. Journal of Agricultural Products Technology, 10(1).
60. Sulistiani & Hidayat, I., 2020. Molecular Identification of Lactic Acid Bacteria from Tempe and Tape Based on the 16S rRNA Gene Sequence. Biosphere Biosphere Scientific Magazine: A Scientific Journal , 37(2), pp. 69-77.
61. Susanto, E. & Fahmi, A. S., 2012. Functional compounds from fish: their application in food. Journal of Food Technology Applications, 1(4), pp. 95-102.
62. Syah, D. P. A., 2014. Analytical hierarchy process method: database software recommendation system. Journal of Informatics, 1(2).
63. Teymoori, F. Golaleh A., Hossein F., Milad N., Mitra A., Parvin M., Fereidoun A., 2019. Various proline food sources and blood pressure: substitution analysis. International Journal of Food Sciences and Nutrition.
64. Utari, D. M., 2010. The content of fatty acids, zinc, and copper in tempeh, how is the potential to prevent degenerative diseases? Indonesian Nutrition, 33(2), pp. 108-115.
65. Vital, R. J. Priscila Z. Bassinello, Quédma A. Cruz, Rosângela N. Carvalho, Júlia C. M. De Paiva, Aline O. Colombo, 2018. Production, Quality, and Acceptance of Tempeh and White Bean Tempeh Burgers. Foods, 7(136).

66. Waziiroh, E. & Istianah, N., 2016. Gluten Free Biscuit Made of Sweet Potato Puree (Ipomea batatas L.) for Autistic Disorder. Jurnal Teknologi Pertanian, 17(2), pp. 79–90.
67. WHO & FAO, 2006. Dalam: L. Allen, B. d. Benoist, O. Dary & R. Hurrell, penyunt. Guidelines on food fortification with micronutrients/edited. Switzerland: World Health Organization and Food and Agriculture Organization of the United Nations.