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Development of biscuits from *bangle* (*Zingiber cassumunar* Roxb.) rhizome flour and purple sweet potato (*Ipomoea batatas* (L.) Lam.) flour, and their potential as antihyperlipidemic functional foods

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Abstract

Zingiber cassumunar Roxb. (ZC) and purple sweet potato (PSP) are the potential sources of antioxidants, and play an important role in reducing blood fat levels, and encourage the development of antihyperlipidemic products, usually in the form of biscuits. Biscuits are favoured by consumers because of their delicious and varied tastes, relatively cheap prices, and complete nutritional content. In the present work, biscuits were made with three formula variations of ZC:PSP ratios namely F1 (0.75:5.25 g), F2 (0.45:5.55 g), and F3 (0.2:5.72 g), and then tested for their physical quality based on Indonesian National Standard (SNI), and hedonic testing to get the best formula, including aroma, colour, texture, and taste. Furthermore, *in vivo* antihyperlipidemic testing was carried out on the best formula. The test was conducted by dividing Sprague-Dawley rats into four groups, namely normal, negative, positive (Nutrive Benecol animal, 3.6 mL/day for 28 days), and the best formula (1.944 g/day for 28 days). All groups were induced with high fat diet (HFD) for 28 days, except normal group. Results showed a decrease in cholesterol, triglyceride, serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT) levels in the normal group with values of 99.09 mg/dL, 90.36 mg/dL, 22.66 U/I, and 39.41 U/I, respectively, as compared to the negative group with values of 195.01 mg/dL, 142.44 mg/dL, 29.05 U/I, and 77.19 U/I, respectively. There was an increase in cholesterol, triglyceride, SGOT, and SGPT levels in the positive group with values of 108.39 mg/dL, 96.12 mg/dL, 24.11 U/I, and 48.55 U/I, respectively, as compared to the normal group test. A combination of biscuits made from ZC and PSP flour could have the potential to reduce lipid levels in the blood, and encourage the development of antihyperlipidemic products.

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Introduction

Hyperlipidaemia is characterised by an increase in one or more plasma lipids, including triglycerides, cholesterols, cholesterol esters, phospholipids, and/or plasma lipoproteins, including very low low-density lipoproteins, accompanied by a decrease in high-density lipoproteins (Shattat, 2014). Hyperlipidaemia can be triggered by an unbalanced lifestyle such as lack of exercise, smoking, and unhealthy eating patterns (Harikumar *et al*., 2013). Management of hyperlipidaemia can be done mainly through behaviour/lifestyle modification, among others, by adopting a low-fat diet, and doing adequate physical activity (exercise). Hyperlipidaemia can be treated with conventional medicines, but natural and traditional medicines have also been developed to treat hyperlipidaemia (Han *et al*., 2021).

Zingiber cassumunar Roxb. (ZC) rhizome is a natural ingredient that contains alkaloids, flavonoids, essential oils, saponins, starches, tannins, and steroids/triflavonoids which have the potential to reduce blood lipid levels (Hermansyah, 2015; Mutia, 2018). ZC rhizome has antioxidant activity associated with hyperlipidaemia; ZC rhizome with 7% curcumin content has been evaluated to affect the activity of superoxide dismutase (SOD) enzyme in rats fed with high-fat diet (HFD) (Han *et al*., 2021). ZC rhizome

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extract could also increase SOD enzyme activity after four weeks, at a dose of 400 mg/kg BW (Sari *et al*., 2020).

Purple sweet potato (PSP; *Ipomoea batatas* (L.) Lam.) contains anthocyanin compounds. Acylated anthocyanins from PSP can serve as natural colorants due to their high heat and light stability (Montilla *et al*., 2011). The anthocyanin compounds have a function as antioxidants and free radical scavengers (Park *et al*., 2011). PSP contains chlorogenic, dicaffeoylquinic, caffeic, and ferulic acids (Jung *et al*., 2011). The anthocyanin content of PSP which is around 30.446 mg/kg DW (dry weight), is found in the outer layer (Im *et al*., 2021).

Antioxidants are associated with antihyperlipidemic. Potential bioactive components of ZC and PSP encourage the development of various antihyperlipidemic functional food products. Functional food is food that can provide health effects for consumers beyond the source of nutrients. This food can be found every day in the community, and includes both food and drink. One type of functional food that is often consumed by the community is biscuits. Biscuits are favoured by consumers for several reasons including delicious and varied tastes, relatively cheap prices, and complete nutritional contents. There are various types and shapes of biscuits on the market. This makes biscuits very attractive.

In previous studies, PSP has been used as locally sourced ingredient in the manufacture of biscuits. But the contents of nutrients such as carbohydrates, proteins, fats, iron, and vitamin C in PSP biscuits were not good enough, so PSP biscuits are more suitable for consumption as an additional food that can meet the daily nutritional needs (Syarfaini *et al*., 2017). Meanwhile, ZC has been used as a functional food in the form of a functional drink that has antioxidant activity (Sunia Widyantari, 2020). Seeing the potential content of the bioactive compound, the combination of ZC and PSP can be used as functional food ingredients, especially in the manufacture of antihyperlipidemic biscuits.

Materials and methods

Plant materials

Zingiber cassumunar (ZC) rhizome flour and purple sweet potato (PSP) flour were obtained from Progress Jogja Yogyakarta, Indonesia. Egg yolk, low fat margarine (Forvita), low fat milk (Lactona), baking powder (Hercules), and refined sugar were obtained from local market.

Preparation of ZC-PSP biscuits

The formulation of ZC-PSP was carried out by preparing three variations of the biscuit formula (F1, F2, and F3) as presented in Table 1. The process of making ZC-PSP biscuits began by putting egg yolks, refined sugar, and margarine into a container and mixing them using a mixer. Then, milk and baking powder were added into the same container, and mixed until homogeneous. After that, ZC rhizome and PSP flour were added little by little until a smooth and easy-to-shape dough was formed. Next step was the moulding process. The dough was moulded and placed on a baking sheet to enter the baking stage. The biscuits were baked in an oven at 120°C for 50 min.

Table 1. Ingredients of different biscuit formula.

Ingredient	F1 (%)	F2 (%)	F3 (%)
Zingiber cassumunar Roxb. (ZC) rhizome flour	5	3	2
Purple sweet potato (PSP) flour	39	41	42
Egg yolk	15	15	15
Margarine (Forvita)	15	15	15
Baking powder (Hercules)	½ tsp	½ tsp	½ tsp
Low-fat milk (Lactona)	4	4	4
Refined sugar	22	22	22

F1, F2, F3: biscuit formula.

Quality evaluation of biscuits

The quality parameters evaluated included the physical properties of the biscuits in the form of thickness, weight, and hardness. Furthermore, proximate analysis was also conducted on moisture, ash, crude fat, crude protein, carbohydrate, and crude fibre contents.

Thickness

Biscuit thickness was determined using a calliper from five biscuits from each formula (F1, F2, and F3) which were stacked on top of each other, measured, and averaged (mm). The test was carried out three times (Wihenti *et al*., 2017).

Weight

Biscuit weight was determined using an analytical scale where five biscuits from each formula (F1, F2, and F3) were weighed separately, and the average weight was calculated. The test was carried out three times (Jauharah *et al*., 2014).

Hardness

Biscuit hardness was determined using a hardness tester, where ten measurements were made with ten different biscuits from each formula (F1, F2, and F3). The test was carried out three times.

Sensory analysis

The biscuits were tested for physical characteristics including colour, aroma, texture, and taste (Table 2). Organoleptic observations on these biscuits were carried out using the sensory evaluation method. Sensory evaluation is a scientific method used to generate, measure, analyse, and interpret the perceived response of a product through the human senses, both objectively and subjectively. Sensory evaluation of colour, aroma, texture, and taste was carried out by a limited tasting panel of three people (Setyaningsih *et al*., 2010).

Table 2. Organoleptic results of different biscuit formula.

Organoleptic attribute	Result		
	F1	**F2**	**F3**
Colour	Brown	Brown	Brown
Aroma	Distinctive	Distinctive	Distinctive
Texture	Crunchy	Crunchy	Crunchy
Taste	Bitter	Somewhat bitter	Somewhat sweet

F1, F2, F3: biscuit formula.

Proximate analysis

Water content

Water content was determined using the SNI standard method (2006). The principle of this analysis is the removal of molecules by heating in an oven at 105°C for 16 h. The determination of water weight is calculated gravimetrically based on the difference in sample weight, before and after the sample is dried. Weight reduction is the amount of water in the material.

Ash content

Ash content was determined using the SNI standard method (2010). The principle of ash content analysis is based on gravimetry. The sample is oxidised or ignited in a kiln at 550°C until white ash is obtained. A dry homogeneous sample of 2 g was put into a porcelain dish, transferred to a furnace, heated at 550°C for 24 h, and weighed.

Crude fat content

Crude fat content was determined using the Soxhlet method. The principle of crude fat analysis using the Soxhlet method is fat extraction with organic solvents such as petroleum ether, petroleum benzene, diethyl ether, acetone, methanol, and so on. The fat is separated from the solvent by evaporating the solvent by heating (Nurcholis, 2013).

Crude protein content

Crude protein content was determined using the formol method (Sudarmadji *et al*., 1989). The principle of formol titration is to neutralise the solution with NaOH base to form dimethylol with the addition of formaldehyde, in which the amino group is already bound, and does not affect the acid-base reaction of NaOH. The indicator used is PP. The final reaction of the titration will be a colour change to pink.

Carbohydrate content

Carbohydrate content was determined using the difference method (carbohydrate content = 100% – (water content + ash content + protein content + fat content) (Sudarmadji *et al*., 1989).

Hedonic test

Assessment of product or hedonic acceptability test requires instruments. The tools used consist of people/groups of people called panels, while people who serve as panels are called panellists. The panellists involved were 20 untrained panellists (aged 20 - 50 years) who were randomly selected and met the criteria, namely being healthy, not sick, not colour blind, not hungry, and willing to participate (Permadi *et al*., 2018). The 20 panellists were trained on their sensory acuity by testing the panellists to find out the introduction to basic tastes, namely sweet, sour, bitter, and umami (savoury).

Panellists were then given a questionnaire sheet with subjective assessments of three variations of biscuit formula, on which organoleptic tests were conducted to analyse colour, aroma, taste, texture, and overall characteristics. The method used was a six-point rating test with scale 6 showing the best trait, and scale 1 the lowest (Setyaningsih *et al*., 2010). The six-point hedonic scale was as follows: 6 = really like; 5 = like; 4 = somewhat like; 3 = neutral; 2 = slightly dislike; and 1 = dislike.

In vivo antihyperlipidemic study

Animals

The experimental test animals were Sprague-Dawley rats weighing 150 - 200 g. To adapt the test animals to their new environment, the animals were acclimatised for 1 w. During acclimatisation, observations were made on the test animals including their body weight and physical condition. All 24 animals were placed in individual cages at 23°C with maintained humidity and 12 h of light and 12 h of dark cycle.

Animal treatment

The Sprague-Dawley rats were divided into four groups. Group 1 = normal group, where the rats were fed with standard feed for 28 d. Group 2 = negative group, where the rats were fed with high-fat diet (HFD) for 28 d. Group 3 = positive group, where the rats were fed with HFD and Nutrive Benecol, 3.6 mL/day for 28 d. Group 4 = test animal group, were the rats were fed with HFD and F3 biscuits at 1.944 g/day for 28 d.

Dosage calculation

Flour dosage

Dosage of Zingiber cassumunar (ZC) flour

Effective dose = 200 mg/kg BW in rats (Sari *et al*., 2020). Dose conversion = 200 mg/kg BW in rats. Rat weighed 200 g = 0.2 kg. Then, the dose administered to rats was 200 mg × 0.2 kg = 40 mg/200 g rats. Rat-to-human conversion factor (70 kg) = 56, then the dose to humans was 56 × 40 = 2,240 mg.

Dosage of purple sweet potato (PSP) flour

Maximum dose based on the results of clinical trials = 160 g/day. Positive control dose (Nutrive Benecol orange): Nutrive Benecol dose per day for humans = two bottles (@100 mL) = 200 mL (Kalbe Farma). Dose conversion factor to convert dose from humans to rats = 200 g = 0.018. Dosage given to rats = 200 mL × 0.018 = 3.6 mL/day. Nutrive Benecol was used as a positive control because the products had clinical trials of cholesterol lowering effect (Kalbe Farma).

Dosage of biscuits

The recommended intake of biscuits formula 3 (F3) per day for humans is eight pieces (@13.5 g) or 108 g. Dosage given to rats was 108 g × 0.018 = 1.944 g/day for rat weighing 200 g. The dose of F3 biscuits was 1,944 g/200-g body weight of rat, and the dose volume given was 4 mL. A stock of 50 mL was made. Next, 50 mL of stock solution were divided by the given volume of 4 mL. Then, the biscuits were weighed 24.25 g. The content of biscuits in the solution was 24.25 g/50 mL × 100% = 48.5%.

High-fat diet (HFD) preparation

High-fat diet (HFD) was prepared by mixing 300 g of standard pellets, 20 g of chicken egg yolks, 100 g of butter, and 10 g of meat fat, and then forming the mixture into pellets, which were then dried. The rats were also given 0.05% propylthiuracil (PTU) in drinking water (Sari *et al*., 2020). Rats were fed 20 g/day and drinking water *ad libitum*. PTU was given to induce an increase in cholesterol.

Standard feed composition

Standard feed composition contained corn, wheat flour, soybean meal (SBM), meat meal, bone meal (MBM), corn gluten (CGM), wheat bran, poultry product flour (PBPM), DDGS, palm oil bran (palm olein), starch acid, sodium bicarbonate, vitamins, and minerals (COMFEED).

Blood sampling

Sampling was carried out on the 29th day. Blood was drawn through the eye vein using a haematocrit tube. Then, the blood was collected and centrifuged at 3,000 rpm for 20 min to obtain blood serum. Next, total cholesterol, triglyceride, SGOT, and SGPT levels were measure using Kit Cholesterol FS, Triglyceride FS, GOT (ASAT) FS, and GPT (ALAT) FS reagents from Diasys®, respectively.

Measurement of total cholesterols, triglycerides, SGOT, and SGPT

The procedure was carried out following the manufacturer’s protocol. Measurement of total cholesterol used the CHOD-PAP method. The CHOD-PAP method or the enzymatic colorimetric method (cholesterol oxidase method) is a method required by WHO/IFCC standards. The principle of the CHOD-PAP method is the determination of cholesterol after enzymatic hydrolysis and oxidation. The reagent used was Kit's reagent (Cholesterol FS, Diasys®).

Triglyceride levels were measured using a colorimetric enzymatic test that employed glycerol-3phosphate-oxidase (GPO). The principle of the GPO method is the measurement of triglycerides after undergoing enzymatic breakdown by lipoproteinase. The reagent used was Kit's reagent (Triglyserida FS, Diasys®).

Measurement of SGOT and SGPT used the UV test method that was optimised following IFCC (International Federation of Clinical Chemistry). The reagents used were GOT reagent (ASAT) FS and GPT reagent (ALAT) FS from Diasys®.

Ethics statement

All procedures involving animals and their care were conducted in accordance with the Guidelines for Care and use of Laboratory Animals, and approved by the Research Ethics Committee of Ahmad Dahlan University (Yogyakarta, Indonesia) with the ethics approval number: 012105028.

Statistical analysis

The data were analysed with SPSS 16.0 version software from United States at a 95% confidence level of 0.05. The test conducted was One-way ANOVA to see the effect of the treatment of each group. There were assumptions that must be met, namely: the observation data were normally distributed, the sample came from an independent group, and the variance between groups was homogeneous. If there was an effect ($p < 0.05$), then it was continued with Tukey's test to find out multiple comparisons in each test group.

Results and discussion

Quality evaluation of ZC-PSP biscuits

The test results of the thickness, weight, and hardness of the biscuits are shown in Figure 1.

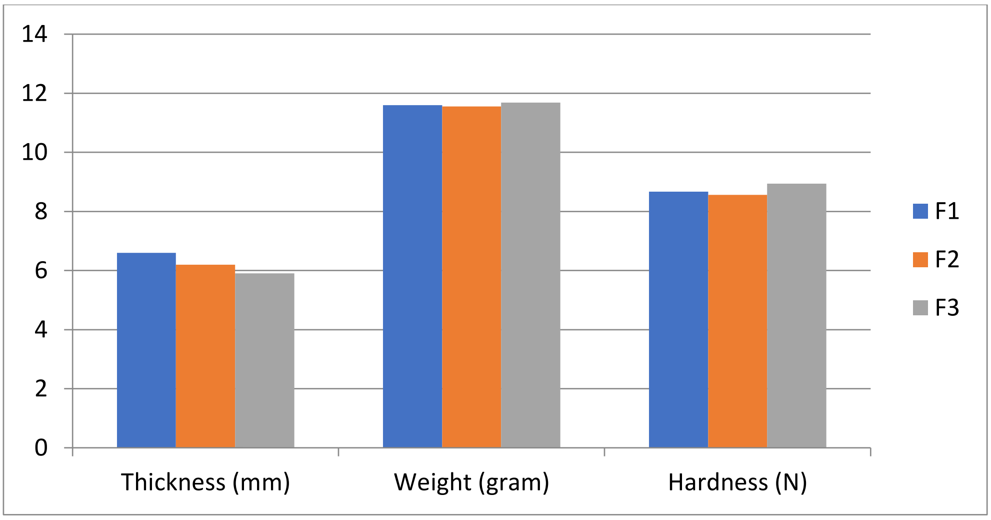

Figure 1. Thickness, weight, and hardness of different biscuit formula. There were no significant differences in the thickness, weight, and hardness of the biscuits for each formula (F1, F2, F3). Thickness = A: Mean (6.6 mm), Std. Dev. (0.01); B: Mean (6.2 mm), Std. Dev. (0.00); C: Mean (5.9 mm), Std. Dev. (0.01). Weight = A: Mean (11.60 g), Std. Dev. (0.02); B: Mean (11.55 g), Std. Dev. (0.02); C: Mean (11.68 g), Std. Dev. (0.03). Hardness = A: Mean (8.67 N), Std. Dev. (0.03); B: Mean (8.56 N), Std. Dev. (0.04); C: Mean (8.94 N), Std. Dev. (0.03).

There was no significant difference in thickness between each formula ($p > 0.05$). The thickness of the biscuits is formed due to the starch heating mechanism so that the starch gelatinisation process occurs which causes the biscuits to expand. Gelatinisation is a gel formation process that begins with the swelling of starch granules due to water absorption during heating. Gelatinisation is a process of breaking down the crystalline form of starch granules so that each surface layer of the molecule can absorb water or dissolve, and react with other materials, because gelatinisation is irreversible, the starch cannot revert to original state (Tako *et al.*, 2014).

The results of the biscuit weight test (Figure 1) showed that there was no significant difference between each formula ($p > 0.05$). Biscuit weight can be affected by the mixing and baking processes. In addition, the accuracy of the composition and mixing time also affect the weight of the biscuits (Agustina, 2019). The weight of the biscuit is also affected by its thickness. The thicker the shape of the biscuit, the heavier the biscuit.

The results of the biscuit hardness test (Figure 1) showed that there was no significant difference between each formula ($p > 0.05$). Hardness is an important indicator, and closely related to biscuit texture. Biscuit hardness is influenced by temperature, baking time, degree of gelatinisation, and degree of development (Mutia, 2018). Starch is another important component of wheat or other flour. Water is bound by starch when gelatinisation occurs, and will be lost during roasting. This causes the dough of the product to become crunchy during baking process. This level of hardness is related to the crunchiness of the biscuits. The higher the hardness, the lower the crunchiness. The level of biscuit hardness is also inversely proportional to the rate of expansion ratio; the lower the swelling ratio, the higher the hardness.

Organoleptic quality of ZC-PSP biscuits

Colour

The results in Figure 2 show that the biscuits met the quality requirements of Indonesia National Standard (SNI) with brown colour, distinctive aroma, and crunchy texture. However, the taste of biscuit F3 showed the best results since it produced unbitter taste. The increase in PSP flour in F3 might have supressed the bitter taste of ZC flour. Based on Figure 2, the resulting colour of biscuits F1, F2, and F3 are brown. This met the requirements for good criteria according to SNI 01-2973-1992, namely the colour of the biscuits should be brown or golden yellow. Fairus *et al*. (2021) produced biscuits from PSP and peanut flour, and also reported brown colour. Colour is the main sensory parameter seen by consumers (Tarwendah, 2017). The brown colour of the biscuits was due to the presence of starch (carbohydrates) and sugar content in the biscuit composition. The brown colour of the three biscuit formula was also caused by non-enzymatic reactions between starch, sugar compounds, and the protein content of the biscuits. The higher the protein and starch/sugar content in the material, the more likely a browning reaction, which causes a lower brightness level (Claudia *et al*., 2015).

Aroma

In Figure 2, it can be observed that the aromas produced by F1, F2, and F3 biscuits were distinctive. This met the requirements for good criteria according to SNI 2973:2011, namely the typical/normal aroma of biscuits. This is in accordance with the research of Fairus *et al*. (2021) on organoleptic testing of the aroma of biscuits made with PSP and peanut flour which produced a distinctive aroma of biscuits. Aroma is one of the important quality factors for consumers' acceptance (Tarwendah, 2017). The distinctive aroma obtained from the biscuits came from the combination of the distinctive aroma of ZC and PSP flours. However, in this case, the aroma of ZC flour was stronger than that of PSP flour. This was due to the volatile oil content of ZC flour. The essential oil content of ZC in dry weight is 1.12 - 3.35% (Rahardjo *et al*., 2004). This causes a distinctive odour on the biscuits. The aroma can also be caused by other ingredients in the dough such as sugar, margarine, butter, egg yolks, milk powder, and leavening agents that function as aroma regulators (Fairus *et al*., 2021). In the food industry, aroma testing is considered important because it can quickly provide an assessment of whether the product is accepted or not. The emergence of this smell or odour is because the odour substance is volatile (evaporated), and slightly soluble in water and fat (Sari *et al*., 2020).

Figure 2. Shape of biscuits of different formula (F1, F2, F3).

Texture

In Figure 2, the resulting texture on F1, F2, and F3 biscuits is crunchy. This is in line with the research of Fairus *et al*. (2021) on organoleptic testing of texture of biscuit made with PSP and peanuts flour, which resulted in a crunchy texture of cookies. The three biscuit formula had a crunchy texture, but the F3 biscuit had a slightly hard texture when it was broken. This is because the highest PSP flour substitution is in F3. PSP flour in F3 contained more amylose. The higher amylose content can cause the texture to be harder because amylose has the property to not easily absorb water, which can affect the resulting texture (Fairus *et al*., 2021). Crispness is one of the parameters in testing biscuit products, and related to the water content. The more water that is evaporated during roasting will form more air cavities, thus resulting in crispier product (Amertaningtyas and Jaya, 2011).

Taste

As presented in Figure 2, the resulting tastes of F1, F2, and F3 biscuits are bitter, slightly bitter, and slightly sweet, respectively. The difference in taste between the three formula was caused by differences in the composition of ZC flour in each formula, wherein ZC had bitter taste. On the other hand, the research of Fairus *et al*. (2021) on organoleptic testing of the taste of biscuits made with PSP and peanut flour discovered that the biscuits produced a sweet taste because the basic ingredients used were PSP and peanut flour which did not have a bitter taste like ZC flour. Taste is an important criterion in assessing a food product that involves a gustatory organ, namely the tongue. Taste comes from a combination of ingredients and composition in a food product that is captured by tongue, and is one of the supporters of taste that supports the quality of a product (Tarwendah, 2017). The quality of the F3 biscuits in the present work met the requirements or good criteria according to SNI 2973:2011, namely the taste of the biscuits should be slightly sweet/normal. As compared to F1, F3 had slightly sweet taste, and was still acceptable. Substitution using PSP flour could mask the bitter taste of ZC flour since PSP flour has a sweet taste.

Proximate composition of ZC-PSP biscuits

Water content

The results of the water content test are shown in Table 3. According to SNI 01-2973-2011, the moisture content of biscuits should not be more than 5%. This means that all formula did not meet SNI quality requirements: F1, F2, and F3 of 7.48, 7.04, and 6.52%, respectively. This could have been due to the high-water content in the flour used (ZC = 9.22%, and PSP = 8.18%). The moisture content of the biscuits decreased with decreasing addition of ZC flour in the formula. This was because the water content in ZC flour was greater than the water content in PSP flour. The research by Izza *et al*. (2019) regarding the determination of the moisture content of biscuits made with PSP and peanut flour using four formula variations with a ratio of F0 (0:0%), F1 (55:20%), F2 (45:30%), and F3 (35:40%) obtained the results of water contents of 5.70, 4.91, 4.70, and 3.97%, respectively. The four water content results from the four variations of the formula met the characteristics of biscuits according to USDA (minimum of 3.57%) and SNI (maximum of 5%). If the two studies are compared, biscuits made with a combination of ZC and PSP flour had higher water content than biscuits made with a combination of PSP and peanut flour. The moisture content of a biscuit is influenced by the basic ingredients of the biscuit formulation. Higher water content in a biscuit base material will affect the value of the moisture content in the produced biscuits. The combination of the two

Table 3. Proximate compositions of different biscuit formula.

Parameter (%)	Result			SNI criteria for biscuit
	F1	F2	F3	
Moisture	7.48 ± 0.07[b]	7.04 ± 0.02[b]	6.52 ± 0.00[b]	Max. 5%
Ash	2.75 ± 0.04[b]	3.14 ± 0.03[b]	3.00 ± 0.06[b]	Max. 1.6%
Fat	18.82 ± 0.13[a]	18.22 ± 0.03[a]	18.30 ± 0.14[a]	Min. 9.5%
Protein	5.84 ± 0.09[b]	6.00 ± 0.22[b]	6.00 ± 0.16[b]	Min. 9%
Carbohydrate	65.09 ± 0.07[b]	65.55 ± 0.20[b]	66.16 ± 0.26[b]	Min. 70%
Fibre	1.70 ± 0.48[b]	1.58 ± 0.28[b]	1.37 ± 0.05[b]	Max. 0.5%

F1, F2, F3: biscuit formula. [a]qualifying for SNI; and [b]not qualifying for SNI.

different flours also affected the moisture content of the biscuits since each flour had different water contents (Izza *et al*., 2019). Moisture content is also affected by humidity, and can be affected by the air temperature of the biscuit storage. The higher the air temperature of the biscuit storage, the higher the moisture in the biscuits.

Ash content

The results of ash content are shown in Table 3. According to SNI 01-2973-2011, the ash content of biscuits should not be more than 1.6%. This means that all formula exceeded the quality standards of F1, F2, and F3 of 2.75, 3.14, and 3.00%, respectively. This could have been due to the high ash content of ZC rhizome flour. ZC rhizome flour contains a fairly high ash content of 6.42%. The high ash content in the ZC rhizome could have affected the ash content in the biscuits. The ash content represents the total mineral content in the biscuit product. The results of previous studies by Mokodompit *et al*. (2017) regarding the determination of the ash content in biscuits made with Goroho banana (*Musa acuminaffe*) and PSP flour using four formula variations with ratio of treatment A (50:50%) revealed an ash content of 2.15%; treatment B (62.5:37.5%) had an ash content of 2.23%; treatment C (75:25%) had an ash content of 2.15%; and treatment D (82.5:12.5%) had an ash content of 2.04%; thus indicating that the results also did not meet the quality standards of SNI. Meanwhile, based on the results of previous research, biscuits that are only made with Goroho banana flour had an ash content that met the SNI quality requirements of 0.02%, and according to the results of Amriani's research (2017), biscuits made with only PSP had ash content which did not far exceed the standard that has been set at 1.79%. This means that the combination of two different types of flour caused the high ash content of the biscuits. The combination of more diverse types of flour in a biscuit formulation will affect the ash content value obtained (Mokodompit *et al*., 2017).

Fat content

The results of fat content are shown in Table 3. According to SNI 01-2973-2011, the minimum biscuit fat content is 9.5%. It means that the fat content contained in the three biscuit formulations showed results in accordance with the SNI quality standard (F1, F2, and F3 of 18.82, 18.82, and 18.30%, respectively). The results of research by Putri *et al*. (2021) regarding the determination of fat content in biscuits made with PSP flour combined with wheat flour with three variations formula revealed that the fat contents were P1, P2, and P3 of 27.680, 27.877, and 28.416%, respectively. Meanwhile, according to the results of previous research by Mokodompit *et al*. (2017) regarding the determination of fat content in biscuits made with Goroho banana and PSP flour using four formula variations, with ratio of treatment A (50:50%) had fat content of 21.72%; treatment B (62.5:37.5%) had fat content of 22.73%; treatment C (75:25%) had fat content of 22.94%; and treatment D (82.5:12.5%) had fat content of 22.32%; thus meeting the SNI quality standards. The fat content of biscuits is also influenced by the fat content of the basic ingredients of biscuits. The higher the fat content in the basic ingredients of biscuits, the higher the fat content in the produced biscuits (Putri *et al*., 2021).

Protein content

The results of protein content are shown in Table 3. According to SNI 01-2973-2011, the protein content of biscuits is at least 9%. This means that the protein content in the three biscuit formula did not meet the quality standards of biscuits (F1, F2, and F3 of 5.84, 6.00, and 6.00%, respectively). Meanwhile, the results of research by Putri *et al*. (2021) regarding the determination of protein content in biscuits made with PSP flour combined with wheat flour with three formula variations reported lower biscuit protein contents, namely P1, P2, and P3 of 1.997, 2.434, and 5.445%, respectively. When they are compared, the protein content results obtained by both studies did not meet the quality standards of SNI for protein content. ZC flour has a protein content of 5.58%, PSP contains a protein of 3.76%, and whole wheat flour has a protein content of 11%. This means that the combination of ZC and PSP flour, and the combination of PSP and wheat flour should be able to achieve a protein content of more than 9%. One of the factors that causes low levels of protein produced in biscuits is protein degradation during the biscuit-baking process which makes a slight decrease in protein levels (Putri *et al*., 2021). Based on previous study, in terms of the effect of heating on the protein content of a swallow's nest, heating for 15 min with an increase in temperature every 100°C from 45 to 95°C decreases protein from 47.56 to 30.88%.

Heating for 30 min, with an increase in temperature every 100°C from 45 to 95°C, decreases protein from 41.69 to 30.5%. The process of baking biscuits with an oven temperature of 120°C can be the cause of the decrease in protein levels in ZC and PSP flour when they are processed into biscuit products.

Carbohydrate content

The results of the carbohydrate content are shown in Table 3. According to SNI 01-2973-2011, the minimum carbohydrate content of biscuits is 70%. This means that the three formula did not meet the quality standards of SNI (F1, F2, and F3 of 65.09, 65.55, and 66.16%, respectively). Meanwhile according to a study by Putri *et al*. (2021) regarding the determination of carbohydrate levels in biscuits made with PSP and wheat flour with three formula variations (P1 = 100:0; P2 = 70:30; P3 = 0:100), they obtained the following results: P1 = 64.454%, P2 = 65.934%, and P3 = 62.015%. The results of both studies did not meet the quality standard of SNI with a minimum standard of 70%. However, the two results were not too far from the standard that has been set (Putri *et al*., 2021). In the present work, the carbohydrate content of the biscuits made with ZC and PSP flours in F3 was higher than the carbohydrate contents in F1 and F2. This was because the composition of PSP flour was higher in F3. ZC flour had carbohydrate content of 69.92%. Meanwhile, PSP flour had fairly high carbohydrate content of 84.92%. The carbohydrate content decreased as the amount of PSP flour was decreased. On the other hand, the more PSP flour that was added, the higher the amount of carbohydrates in the biscuits.

Crude fibre content

The results of crude fibre content are shown in Table 3. According to SNI 01-2973-2011, the maximum biscuit fibre content is 0.5%. The fibre content contained in the biscuits exceeded this (F1, F2, and F3 of 1.70, 1.58, and 1.37%, respectively). However, F3 biscuits was not too far from the standard that has been set. The results of previous studies by Mokodompit *et al*. (2017) on the determination of fibre content in biscuits made with Goroho banana and PSP flour using four formula variations with ratio of treatment A (50:50%) had fibre content of 2.24%; treatment B (62.5:37.5%) had fibre content of 2.88%; treatment C (75:25%) had fibre content of 2.23%; and treatment D (82.5:12.5%) had fibre content of 2.16%; thus indicating that they did not meet the quality standards o SNI. The factor that caused high content of crude fibre in biscuits could be the high content of crude fibre in each flour used as the basic ingredient of biscuits. In the present work, PSP flour had a high fibre content of 4.72%. Therefore, it caused high levels of crude fibre in biscuits to exceed the standard limits that have been set. However, fibre content has an important role to maintain health. According to Kusharto (2006), the fibre in food commonly referred to as dietary fibre is very good for human health. High fibre intake can provide many benefits for the body. Dietary fibre is also a reference for maintaining a healthy digestive tract and general health. The recommended daily dietary fibre intake is 30 g/day. According to BPOM No. 13 (BPOM, 2018), fibre-rich foods contain 6% dietary fibre content; so, all biscuit formula met the requirements for fibre-and nutrient-rich foods.

Hedonic test of ZC-PSP biscuits

Aroma

Based on the results of the hedonic test on the aroma of biscuits using SPSS for windows version 16, the One-way ANOVA test showed significant results, namely 0.001 < 0.05, so it was continued with Duncan's test with 95% confidence. Duncan's test results showed that there was a significant difference between the average values of the three formula (Table 4).

Table 4. Hedonic results of different biscuit formula.

Formula	Aroma	Colour	Texture	Taste	Overall
F1	2.65*	3.75*	4	2.45*	3*
F2	3.25*	4.15*	4.2	3.2*	3.6*
F3	4.1*	4.4*	4.35	4.7*	4.7*
p	**0.001**	**0.000**	**0.000**	**0.043**	

*Significant ($p < 0.05$). F1, F2, F3: biscuit formula.

The highest level of preference for the aroma of biscuits was F3 biscuits. Therefore, it can be concluded that the F3 biscuit gave the best results in hedonic testing. The aroma of biscuits with F3 was most preferred because of the composition of ZC flour which was the lowest among the three formula, so the distinctive odour or aroma of the essential oil contained in ZC flour was not too strong as compared to the aroma of the biscuits with F1 and F2. Then, the PSP flour content was higher in F3 as compared to PSP flour in F1 and F2, so the smell or distinctive aroma of sweet potato from PSP flour was most favoured by the panellists. The aroma can be caused by other ingredients in the dough such as sugar, margarine, butter, egg yolks, milk powder, and leavening agents that function as aroma regulators.

Colour

Based on the results of the analysis of preferences on the colour of biscuits using SPSS for windows version 16, the One-way ANOVA test was found to be significant, namely 0.000 < 0.05, so it was continued with Duncan's test with 95% confidence. The results of Duncan's test showed that there was a significant difference between the F1 with F2 and F3 biscuits (Table 4). Furthermore, there was no significant difference between F2 and F3 biscuits. So, it can be concluded that the colour of F2 and F3 biscuits gave good results in hedonic testing. According to a study by Fairus *et al*. (2021) on the substitution of wheat flour with PSP and peanut flour in biscuit production, the brown colour of the biscuits was favoured by the panellists in biscuit colour testing. The study used four variations of the formula of PSP and peanut flour, namely F0 = 0:0% which showed a golden yellow colour with a hedonic scale value of 4.2 (very like); F1 = 55:20% which showed a brownish colour with a hedonic scale value of 3.4 (like); F2 = 45:30% which showed a brownish colour with a hedonic scale value of 3.5 (like); and F3 which showed a light brown colour with a hedonic scale value of 3.6 (like). The brown colour of the biscuits is due to the Maillard reaction. Maillard reaction is a reaction between carbohydrates that produces hydroxymethylfulfural compounds. Furfural polymerises to form brown melanoidin compounds and (Fairus *et al*., 2021).

Taste

Based on Figure 2, the resulting tastes of biscuits F1, F2, and F3 were bitter, slightly bitter, and slightly sweet, respectively. The difference in taste between the three formula was caused by differences in the composition of ZC flour in each formula, wherein ZC flour had bitter taste and the composition of ZC flour was the highest in F1. Fairus *et al*. (2021) on organoleptic testing of the taste of biscuits made with PSP and peanut flour, reported sweet taste on the biscuits because the basic ingredients used were PSP and peanut flour which did not have bitter taste like ZC flour. The quality of the F3 biscuits in the present work met the requirements for good criteria according to SNI 2973:2011, namely the taste of the biscuits that was slightly sweet/normal. As compared to F1, F3 had slightly sweet taste, and was still acceptable because it contained the least amount of ZC.

Texture

Based on the results of the analysis of preference for biscuit texture using SPSS for windows version 16, the One-way ANOVA test was found to be significant, namely 0.000 < 0.05, so it was continued with Duncan's test with 95% confidence. Duncan's test results showed that there was an insignificant difference between the average values of F1 and F2 biscuit textures, while there was a significant difference between the hedonic test results of F1 and F3 biscuit textures. Then, there was an insignificant difference between the results of the hedonic test of biscuit textures of F2 and F3 (Table 4). Based on this, it can be concluded that the biscuit texture of the three formula gave good results in hedonic testing. A study conducted by Fairus *et al*. (2021) regarding the substitution of wheat flour with PSP and peanut flour in the manufacture of cookies revealed that on biscuit texture testing, the crunchy texture of the biscuits was favoured by the panellists. The study used four formula variations for PSP and peanut flour, namely F0 = 0:0% which showed the results of a crunchy texture with a hedonic scale value of 3.9 (like); F1 = 55:20% which was indicated to have a slightly hard crunchy texture with a hedonic scale value of 3.0 (rather like); F2 = 45:30% which was indicated to have a crunchy texture with a hedonic scale value of 3.4 (like); and F3 = 35:40% which showed a crunchy texture with a hedonic scale value of 3.7 (like). Based on these results, the panellists liked the crunchy taste of the biscuits. The protein content in the basic ingredients of biscuits has a high absorption capacity (hydrophilic properties), which can cause the biscuits to become dense and crunchy, and even become hard and less crunchy. Carboxyl groups in protein cookies cause water absorption. If the formula contains high protein, the biscuit texture tends to be harder (Fairus *et al*., 2021).

Overall

Based on the results of the analysis of preference for all biscuits using SPSS for windows version 16, the One-way ANOVA test was found to be significant, namely 0.000 < 0.05, so it was continued with Duncan's test with 95% confidence. The results of Duncan's test showed that there was a significant difference between the three formula, and it could be concluded that the F3 biscuit gave the best results in the hedonic test (Table 4). Overall, the F3 biscuit was the most acceptable. Next, *in vivo* testing was carried out on the best biscuit formulation with measurement parameters for total cholesterol, triglyceride, SGOT, and SGPT levels in rats fed with a high-fat diet (HFD).

Effect of ZC-PSP biscuits on total cholesterol levels in rats fed with HFD

The results of measurements of total cholesterol, triglyceride, SGOT, and SGPT levels can be seen in Table 5. Based on Table 5, it can be seen that there is a significant difference between the test and the negative groups. The results of the SPSS analysis showed that there was an effect of giving biscuits on reducing triglyceride levels in the test group. This was proven in the One-way ANOVA statistical test where the significant value was 0.000 ($p < 0.01$), which meant that H_0 was rejected, and H_a was accepted. The average of all treatments was significantly different, so the results of measuring total cholesterol levels were continued with Tukey's HSD test. In Tukey's test, the results obtained were significantly different. Furthermore, the homogeneity test of triglyceride data was carried out which obtained a significant value of 0.317 ($p > 0.05$), which meant that the variation of the data was homogeneously distributed.

Table 5. Total cholesterol, triglyceride, SGOT, and SGPT of high-fat diet rat fed with different biscuit formula.

Group	Cholesterol level (mg/dL)	Triglyceride level (mg/dL)	SGOT activity (U/I)	SGPT activity (U/I)
Normal	81.75 ± 2.63#	74.56 ± 2.48#	18.21 ± 0.75#	37.87 ± 1.37#
Negative	195.01 ± 3.14*	142.44 ± 4.73*	29.05 ± 1.47*	77.19 ± 0.51*
Positive	108.39 ± 4.91#*	96.12 ± 2.42#*	24.11 ± 1.16#*	48.55 ± 0.51#*
Treatment	99.09 ± 2.63#*	90.36 ± 2.04#*	22.66 ± 0.75#*	39.41 ± 0.75#*

#Significant differences ($p < 0.05$) with negative controls. *Significant difference ($p < 0.05$) with normal controls. Normal control: rats fed with standard for 28 d; Negative control: rats fed with high-fat dietary (HFD) for 28 d; Positive control: rats fed with HFD and Nutrive Benecol 3.6 mL/day for 28 d; Treatment: rats fed with HFD and F3 biscuits 1.944 g/day for 28 d.

A HFD caused increasing cholesterol in negative group. This was due to the continuous feeding of HFD for 28 d in the negative group. HFD intake can lead to high concentrations of LDL cholesterol (bad cholesterol). Fat content, especially saturated fat, increases LDL levels by decreasing the synthesis and activity of LDL receptors. Saturated fat is the main cause of an increase in LDL because if there is an increase in saturated fat, it will reduce the activity of LDL uptake by LDL receptors, and reduce cholesterol excretion in the blood vessels. The lack of LDL receptors causes LDL not to be captured by LDL receptors. As a result, LDL levels will increase, thus making it longer in circulation, and more likely to be oxidised. It is this oxidised LDL that is highly atherogenic. F3 biscuits affected reducing total cholesterol levels in the test control group. This could have been due to the content of ZC and PSP flours which have the potential as antihyperlipidemic agent.

ZC is one of the natural ingredients that are traditionally used to lower fat. According to Paramita (2018), the content of ZC which is useful for lowering cholesterol levels is curcumin, which has antioxidant activity, and plays a role in reducing lipids. The antioxidant mechanism of curcumin is related to the presence of the H atom in the phenolic group. The action of curcumin as a radical scavenger is that it can maintain the integrity of cell membranes during oxidative degradation events due to the presence of oxygen radicals and other reactive radicals (Mahfudh *et al*., 2021).

Curcumin content of 7% in ZC rhizome has been evaluated to affect the activity of the enzyme superoxide dismutase (SOD) in rats fed with HFD (Han, 2021). According to Hermansyah (2015), there are 33.2 mg of curcumin in 1 g of ZC rhizome extract. In F3 biscuits, there were 0.28 g of ZC rhizome in each biscuit chips. The provision of biscuits per day was eight pieces per day, so there were 2.24 g of ZC rhizome given to the test control group. In 2.24 g of ZC rhizome flour, it was estimated that there were about 60 mg of curcumin. Fat metabolism will cause the production of ROS to increase which causes oxidative stress. Oxidative stress can be characterised by a decrease in SOD. The content of curcumin in ZC rhizomes will increase the activity of SOD, and restore the oxidative balance (Mahfudh *et al*., 2021). In addition to curcumin, the rhizome of ZC also contains flavonoid compounds that have antioxidant activity. Based on previous study, flavonoid compounds can inhibit cholesterol absorption, increase bile excretion, and inhibit 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). Flavonoids are also known to have strong activity in inhibiting lipid peroxidase by inhibiting the activity of acyl-coenzyme:cholesterol acyltransferase (ACAT) which plays a role in esterification of cholesterol in the intestine and liver, and inhibits the activity of the 3-hydroxy-3-methyl-glutaryl-CoA enzyme which results in the inhibition of cholesterol synthesis. Other phenolic content, such as tannins, are also involved in lowering cholesterol levels through inhibition of lipid absorption.

ZC has moderate antioxidant activity with an IC_{50} value of 142 mg/mL. Antioxidant compounds can lower cholesterol levels in the blood by preventing LDL oxidation. The antioxidant content in ZC rhizomes fights free radicals of reactive oxygen species through the inhibition of oxidation. Oxidation inhibition occurs through reactions that will convert reactive free radicals into relatively stable unreactive free radicals (Romadhoni *et al*., 2014). ZC rhizome contains phenolic compounds, and the total phenolic content of ZC rhizome is 2.71 g GAE/100 g extract or 0.30 g GAE/100 g simplicia. *In vivo* research using 30 rats given ZC rhizome extract at a dose of 400 mg/kg showed a significant reduction in total cholesterol (Mahfudh *et al*., 2021).

PSP also has antioxidant activity that can reduce the amount of cholesterol in the blood (Yunarto, 2019). PSP contains anthocyanin compounds which are natural dyes from the flavonoid group. According to Priska *et al*. (2018), the antioxidant function of anthocyanins has various benefits in preventing various degenerative diseases, for example, cardiovascular disease due to atherosclerosis, by inhibiting and reducing cholesterol levels in the blood caused by LDL oxidation. In other words, anthocyanins can protect fat cell membranes from oxidation. Cholesterol levels lowered by anthocyanins in this case reached up to 13.6% when anthocyanins were consumed for ≥ 12 weeks with an average consumption of anthocyanins in women around 19.8 - 64.9 mg/day, and in men around 18.4 - 44.1 mg/day (Priska *et al*., 2018). In F3, there were 5.72 g of PSP in each biscuit chip. The provision of biscuits per day was eight pieces per day so that there were 45.76 g of PSP flour given to the test control group. According to Husna and Novita (2013), there is anthocyanin content of 61.85 mg/100 g of PSP. So, it was estimated that there was anthocyanin content of 28.30 mg in F3 which had activity in lowering cholesterol levels in the control test. Anthocyanins lower cholesterol through the mechanism of breaking the chain of propagation of free radicals where all hydroxyl groups (OH) in ring B can donate or act as electron or hydrogen donors so that free radicals are scavenged or intercepted (Priska *et al*., 2018).

In addition to the contents of ZC rhizome and PSP, the high fibre content in the biscuits also caused the decrease in cholesterol in the test animals. Soluble fibre can trap fat in the small intestine, so fibre can lower cholesterol levels in the blood by 5% or more. In the digestive tract, fibre can bind to bile salts (the end product of cholesterol) which is then excreted together with faeces. Therefore, dietary fibre can reduce cholesterol levels in blood plasma. When there is an increase in the excretion of cholesterol in the faeces, it will reduce the amount of cholesterol that goes to the liver. A decrease in the amount of cholesterol in the liver will increase the uptake of cholesterol in the blood which will be synthesised to become bile acids. This is a factor in the reduction of cholesterol levels in blood plasma.

Based on this, it can be concluded that the decrease in cholesterol levels by F3 biscuits could have been due to the presence of curcumin and flavonoids in ZC rhizome flour and anthocyanin compounds in PSP, because all these compounds have antioxidant activity, and have the potential to reduce lipid levels in the blood. In addition, the fibre content in the biscuits also caused the decrease in cholesterol in the control test.

Effects of ZC-PSP biscuits on triglyceride levels in rats fed with HFD

Based on Table 5, it can be seen that there is a significant difference ($p < 0.05$) between the test and the negative groups. The results of the SPSS analysis showed that there was an effect of giving biscuits on reducing triglyceride levels in the test control group. This was proven in the One-way ANOVA statistical test where the significant value was 0.000 ($p < 0.01$), which meant that H_0 was rejected, and H_a was accepted that the average of all treatments was significantly different. So, the results of the triglyceride level measurement were continued with

Tukey's HSD test. In Tukey's test, the results obtained were significantly different. Furthermore, the homogeneity test of triglyceride data was carried out which obtained a significant value of 0.361 ($p > 0.05$), which meant that the variation of the data was homogeneously distributed.

F3 biscuits had an effect on reducing triglyceride levels in the test group. This is due to the contents contained in ZC rhizome flour and PSP flour in biscuits, which have potential as antihyperlipidemic agents. ZC rhizome contains curcumin, flavonoid, and tannin (Yunarto, 2019). According to Yunarto (2019), curcumin was most effective in lowering triglycerides through the inhibition of lipase. Overall, curcumin affects the same mediators of plasma lipid changes as statins. Almost all cholesterol transport pathways that take place in the body are affected by these compounds. Transport pathways include gastrointestinal absorption of dietary cholesterol, transfer of hepatocellular plasma cholesterol, mediators of reverse cholesterol transport, and transport of cholesterol from peripheral tissues. In addition, the potential for ROS of curcumin limits the risk of lipid peroxidation that triggers an inflammatory response that leads to cardiovascular disease and atherosclerosis. Other phenolic content such as flavonoids and tannins are also involved in decreasing triglyceride levels through the inhibition of lipid absorption (Mahfudh *et al*., 2021).

According to Mutia (2018), antioxidant compounds such as flavonoids are thought to reduce triglyceride levels in rats with hypercholesterolemia. Flavonoid compounds can reduce triglyceride levels by increasing the activity of the LPL (lipoprotein lipase) enzyme. LPL converts VLDL into LDL so that the accumulation of VLDL can be reduced. The mechanism of flavonoid can reduce triglyceride levels through increased LPL enzyme activity, and with the increased VLDL enzyme that transports triglycerides, they will undergo hydrolysis into fatty acids and glycerol. The liberated fatty acids will be absorbed by muscles and other tissues and, then oxidised to produce energy, and adipose tissue will store them as energy reserves. Flavonoid compounds can inhibit fatty acid synthase (FAS), which is an enzyme that is very important in fat metabolism. Inhibition of FAS can directly reduce the formation of fatty acids, thereby reducing the formation of triglycerides (Mutia, 2018).

Tannins also affect the reduction of triglyceride levels in the blood by reducing the absorption of cholesterol and triglycerides in the small intestine, and increasing the excretion of bile acids. The mechanism of tannins as anti-hypercholesterolemic agent is by inhibiting adipogenesis, and inhibiting absorption in the intestine. Tannins are also antioxidant compounds that act as anti-free radicals, and activate antioxidant enzymes. Tannins can prevent the oxidation of LDL cholesterol, reduce body fat, and reduce the incidence of cardiovascular disease (Mutia, 2018).

PSP contains many anthocyanin compounds such as cyanidin, pelarginidin, peonidin, delphidin, petunidin, and malvidin which have antioxidant activity. The mechanism related to the decrease in triglyceride levels by anthocyanins is that anthocyanins inhibit cholesterol synthesis by activating adenosine monophosphate-activated protein kinase (AMPK) which plays a role in energy homeostasis. AMPK works to block HMG-CoA reductase which plays a role in cholesterol synthesis so that AMPK activation will reduce cholesterol synthesis. AMPK also reduces the activity of acetyl Co-A carboxylase which will result in an increase in fatty acid oxidation, and a decrease in fatty acid synthesis, thus resulting in a decrease in cholesterol concentration (Graf *et al.*, 2013).

In addition, the decrease in triglyceride levels was also influenced by the high fibre content in biscuits. A high-fibre diet can have an effect on lipid metabolism. High fibre consumption can reduce total triacylglyceride, total cholesterol, and LDL concentrations, and increase HDL concentrations in blood serum and liver (Affandi and Ferdiansyah, 2017). Based on this, it can be concluded that the decrease in triglyceride levels by F3 biscuits was caused by the presence of curcumin, flavonoids, and tannins in ZC rhizome flour, anthocyanin compounds in PSP, and high fibre content in biscuits.

Effects of ZC-PSP biscuits on SGOT-SGPT levels in rats fed with HFD

Table 5 shows that there is an effect of giving biscuits on decreasing levels of serum glutamic oxaloacetic transaminase (SGOT) in the test control group (F3 biscuits) with an average value of 22.66 U/I. Meanwhile, there was an increase in SGOT levels in the control group (HFD) with a value of 26.05 U/I. The results obtained showed a significant difference, and were proven in the One-way ANOVA statistical test where a significant value of 0.000 ($p < 0.01$) was obtained, which meant that H_0 was rejected, and H_a was accepted; that the average of all treatments was significantly different. So, the results of the measurement of levels triglycerides were continued with Tukey's HSD test. In Tukey's test, the results obtained were significantly different. Furthermore, the homogeneity test of SGOT data was carried out, and obtained a significant value of 0.23 ($p > 0.05$), which meant that the variation in the data was homogeneously distributed.

Table 5 also shows that there is an effect of giving biscuits on decreasing levels of serum glutamic pyruvic transaminase (SGPT) in the test control group (F3 biscuits) with an average value of 39.41 U/I. Meanwhile, there was an increase in SGPT levels in the control group (HFD) with a value of 77.19 U/I. The results obtained show a significant difference, and were proven in the One-way ANOVA statistical test where a significant value of 0.000 ($p < 0.01$) was obtained, which meant that H_0 was rejected, and H_a was accepted; that the average of all treatments is significantly different. So, the results of the measurement of levels triglycerides were continued with Tukey's HSD test. In Tukey's test, the results obtained were significantly different. Furthermore, the homogeneity test of SGPT data was carried out, and obtained a significant value of 0.188 ($p > 0.05$), which meant that the variation of the data was homogeneously distributed.

HFD had an effect on increasing levels of SGOT and SGPT in the negative group. Meanwhile, the administration of F3 biscuits along with HFD showed a decrease in the levels of SGOT and SGPT in the test group. This proved that there was a relationship between hyperlipidaemia and liver function.

Increased cholesterol and triglycerides can increase SGOT and SGPT in the blood. This is because the accumulation of fatty liver can increase fatty acids in the liver that exceeds its oxidative capacity. This will trigger the formation of free radicals that can cause hepatocyte necrosis and other liver damage, which will increase the activity of SGOT and SGPT in the blood. The accumulation of free fatty acids will trigger the action of mitochondria to maintain the stability of hepatocyte cells. Continuous accumulation will cause mitochondrial damage that ends with the accumulation of free radicals in liver tissue. The accumulation of free radicals will cause cell damage and eventually cell death.

Elevated SGOT and SGPT is a sign of liver damage. Increased fatty acids in the blood will be carried to the liver to undergo the oxidation process. Excess fatty acids are stored in the form of triglycerides. The accumulation of triglycerides will increase TNF-α followed by an increase in nicotinamide adenine dinucleotide phosphate (NADPH) oxidase which will produce superoxide radicals. These radical species are highly reactive to lipids, and cause lipid peroxidation. Lipid peroxidation will cause changes in liver cell permeability causing enzymes produced by liver cells, especially SGOT and SGPT to increase in the blood (Graf *et al*, 2013).

ZC contains flavonoid compounds. According to Ramadhani *et al*. (2017), flavonoids as antioxidants will act as free radical carriers. Flavonoids can interact with free radicals using various enzyme systems. Flavonoids are phenolic compounds that can capture free radicals that enter the body. Flavonoids work by binding or forming chelates that will convert free radicals into non-toxic compounds so that free radicals cannot damage the liver. Flavonoids can also inhibit the activity of cytochrome P450 enzymes and their metabolites. Flavonoid compounds can prevent oxidative stress that occurs in the liver. Flavonoids will bind free radicals from alcohol metabolism, prevent lipid peroxidation, and prevent liver damage (release of SGPT and SGOT).

PSP contains anthocyanin compounds that function as antioxidants. According to Sutirta-Yasa *et al*. (2011), administration of ethanolic extract of PSP in test animals at a dose of 1 mg/day given with alcohol at a dose of 0.8 g/day by mouth for 4 w can reduce blood sugar levels. SGOT and SGPT were significant ($p < 0.05$). This was due to the anthocyanin content in PSP. In the present work, it was proven that PSP can protect liver tissue and reduce oxidative stress. Anthocyanins found in PSP function as antioxidants. Anthocyanins are certain flavonoids that can neutralise free radicals in liver tissue.

Based on the measurement results of SGOT and SGPT, it can thus be concluded that hyperlipidaemia greatly affects liver function. Hyperlipidaemia is closely related to liver function. Liver plays an important role in the transport and metabolism of fats, including the production of bile for the excretion of cholesterol, and has an enzyme system that can synthesise and oxidise fatty acids, convert fatty acids into bile acids, and play a role in lipoprotein metabolism. Damage and toxic effects on the liver can interfere with the metabolism and excretion of cholesterol from the body (Yunarto, 2019).

Conclusion

Zingiber cassumunar (ZC) flour which was combined with purple sweet potato (PSP) flour was formulated into antihyperlipidemic biscuit product. The quality the biscuits met Indonesian National Standard (SNI) quality requirements on fat content and organoleptic properties (colour, odour, taste, and texture). The overall hedonic test results showed that F3 biscuit was the most preferred/chosen one. The results of the *in vivo* test with F3 biscuit showed a significant reduction in cholesterol, triglycerides, serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT) with $p < 0.05$ as compared to the negative group. However, biscuit formulations did not meet the quality requirements of SNI for water, ash, protein, carbohydrate, and fibre contents, but could reduce lipid levels in the blood. Based on these conclusions, it is necessary to conduct further studies on biscuit formulations, especially on the physical properties and contents of biscuits, in order to produce good-quality products in accordance with the quality requirements of SNI.

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