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CHEMICAL PROPERTIES AND ACCEPTANCE IN THE BISCUIT FORMULA OF BELITUNG TARO (*XANTHOSOMA SAGITTIFOLIUM)* WITH ADDITION OF ANT NEST TUBERS (*HYDNOPHYTUM FORMICARUM)* PLANT

Anjar Briliannita, La Supu

ABSTRACT
This study was aimed to analyze the nutrients content, antioxidant activity, and acceptance of the biscuit formula of Belitung taro (X*anthosoma sagittifolium*) with various parts of ant nest tubers (*Hydnophytum formicarum*) plant. Antioxidant activity was found by the DPPH method, nutrient content by the AOAC method, and sensory evaluation analysis by 30 untrained panelists. The results of this study found out that each biscuit formula (35.25 grams/73.60% antioxidant activity) contained 130 kcal of energy, carbohydrates of 59.08 ±0.16%, the protein of 4.13 ±0.06%, fat of 22.17 ±0.19% so that it was able to compensate for 5.97 – 6.57% of total energy requirements based on the Nutrition Adequacy Rate for the Elderly per day. The overall preference level in the biscuit formula by untrained panelists was 4.10 ±0.72 (rather like-like). The addition of 7.5% ant nest tuber powder in the biscuit formula had an antioxidant activity (DPPH) of 73.60 ±0.36%. The most preferred biscuit formula was the C013 biscuit formula (addition of 6% ant nest tuber powder per gram biscuits), with the higher antioxidant activity, and its nutrient content to compensate for the nutrition adequacy rate for the elderly per day.

Keywords: biscuits; Belitung taro; ant nests tuber; antioxidant activity; organoleptic test

INTRODUCTION
Belitung taro or kimpul *(Xanthosoma sagittifolium*) is a tropical plant derived from *Araceae* family, containing high starch ranging between 22% to 40%, and because of that, Belitung taro or kimpul is a good source of carbohydrate **(Pérez, Schultz and de Delahaye, 2005**). Small starch granules from Belitung or taro *(Xanthosoma sagittifolium*), from the aspect of digestibility, starch with a small granule size was easier to digest. Other significant nutritional content in Belitung or kimpul taro *(Xanthosoma sagittifolium*) was protein one level higher than sweet potato and cassava (every 100 grams of taro or Belitung *(Xanthosoma sagittifolium*) contains 1.9 g of protein while cassava contains 1.2 g and sweet potato 1.8 g. Belitung taro tubers contain 0.32 g total phenolics per 100 grams of material; 0.26 g flavonoids per 100 grams of material and activity *scavenging* better using DPPH testing (78.22 ±0.56%), *hydroxyl radical* (69.11 ±0.21%), superoxide radicals (83.27 ±0.08%), and ABTS *radical cations* (76.11±0.07%) **(Nishanthini and Mohan, 2012)**.

Meanwhile, *Hydnophytum formicarum* or ant nests tuber plant from West Papua were used in research as a source of antioxidants. This plant was hereditarily consumed in the form of boiled tuber powder and was used to cure diseases such as cancer, tumors, gout, migraine, periodontitis, coronary heart disease, tuberculosis, and leukemia, but it was also used to treat diabetes mellitus. Significantly, the ethyl acetate extract *Hydnophytum formicarum* was also the most potent antioxidant, showing 83.31% of radical scavenging activity with IC_{50} 8.40 μg.mL^{-1} in the DPPH assay. The other extracts display weak to moderate antioxidative activities, ranging from 28.60 – 56.80% radical scavenging. The SOD assay shows that methanol extract exhibits the highest activity (74.19% inhibition of superoxide radical). The dichloromethane and ethyl acetate extracts display comparable SOD activity. The promising bioactivities of the crude ethyl acetate extract guided the first isolation of bioactive flavonoid and phenolic compounds: isoliquiritigenin, protocatechualdehyde, butin, and butein from this species, we propose that *Hydnophytum formicarum* Jack. can serve as a new source enriched with potent antioxidative and antimicrobial agents **(Prachayasittikul et al., 2008)**.

Based on another study *H. formicarum* tuber from Setiu wetland and Muara Rupit showed very good potency as antibacterial and antioxidant agents**.** All samples showed high DPPH free radical scavenging activity (IC50 less than 10 μg.mL^{-1}). Via MTS assay, no cytotoxic activity of all samples was observed against HeLa cells. Only a fraction from Setiu Wetland showed very strong cytotoxic activity against MCF-7 cells (IC50 = 2 μg.mL^{-1}) and its morphological features stained by Annexin-V/PI and DAPI

a

b c

Figure 1 *Hydnophytum formicarum* and ant nest tuber powder
Note: a) ant nest tuber plant (*Hydnophytum formicarum*) raw; b) ant nest tuber plant (*Hydnophytum formicarum*) dry; c) ant nest tuber powder plant (*Hydnophytum formicarum*).

showed that the cell death was mediated by apoptosis. **(Andriani et al., 2017)**.

Studies were conducted on the structure and rheological properties of three cocoyam varieties *Xanthosoma sagittifolium* (red-flesh), *Xanthosonia sagittifolium* (white-flesh) and *Colocasia esculenta* starches and raphides in an attempt to characterize them. No distinct variation was observed in starch granule sizes of the two *Xanthosoma* species. Starch granules sizes in the ranges of 0.74 – 1.19 and 0.74 – 1.10 µm were obtained for the *Xanthosoma* species (red tubers) and *Xanthosoma* species (white tubers), respectively. Significantly smaller sizes (0.05 – 0.08 µm) of starch granules were observed for *Colocasia esculenta*. Peak viscosity was highest in the *X. sagittifolium* (red-flesh) variety while the white-flesh variety showed the least tendency to retrogradation **(Sefa-Dedeh and Kofi-Agyir Sackey, 2002)**. Based its study *X. sagittifolium* species white tubers have a low glycemic index because of their high amylose starches. The glycemic index has been categorized by Miller as low GI is pounds

a b

Figure 2 Beiltung taro and flour.
Note: a) Belitung taro *xanthosoma sagittifolium* raw; b) Belitung taro *xanthosoma sagittifolium* flour.

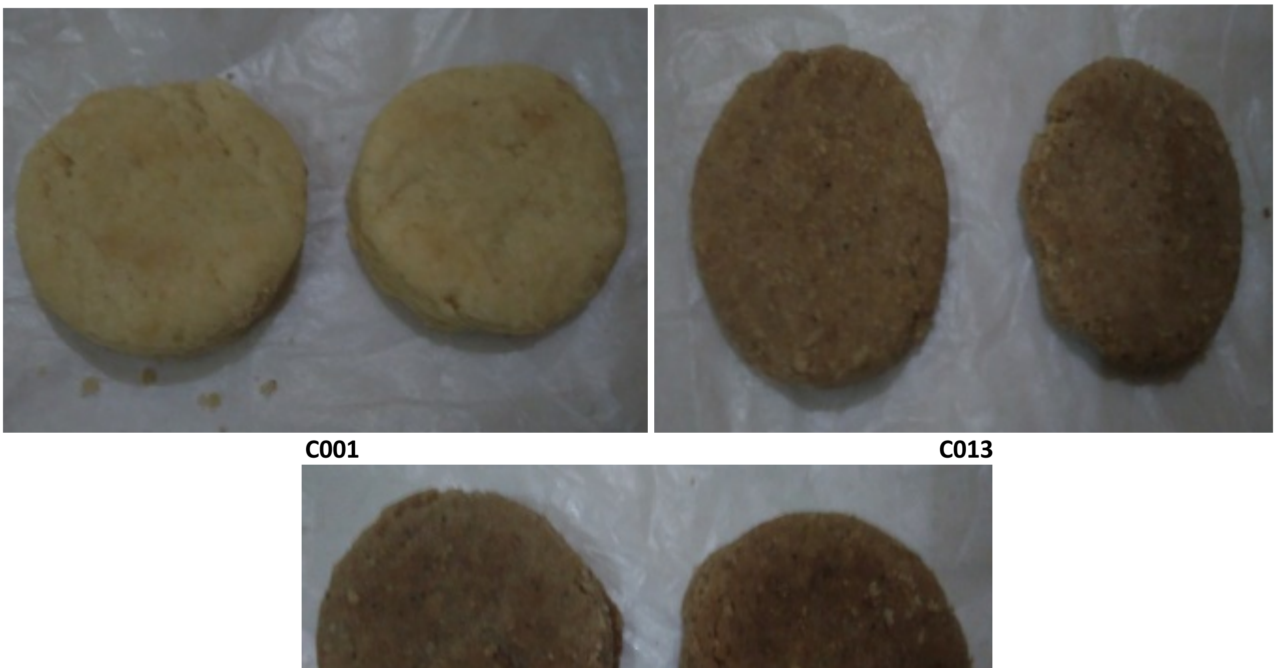

Figure 3 Biscuits made with addition of Beiltung taro flour and ant nest tuber flour.

55, moderate GI is between 56 – 69 and high GI is ≥70. Glycemic index (GI) has been widely used in the management of blood sugar levels among diabetes, however; in the South Pacific, very little information regarding the GI of local foods is made available. Other research was to determine the glycemic index and the glycemic load of 5 South Pacific foods (Plantain (*Musa AAB*), tannia (*Xanthosoma sagittifolium*), roti or chappati, homemade pancake, and Lees cabin crackers) have moderate GI values ranging from 59 to 68. The Glycemic Load (GL) for cabin biscuit was the highest **(Lako et al., 2004)**. Based on sensory test the best treatment was obtained from the proportion of *Xanthosoma sagittifolium* flour: black soybean flour at 70:30 with 2% agar was liked by panelists **(Kasih and Murtini, 2017)**. Biscuits are an instant food that is consumed mostly because of the appetizing, ready to be consumed, the cost is also cheap and the availability of short time and biscuits can make a significant contribution to the daily intake of cereals. However, in an era of increasing functional food popularity, new demands have been set for various categories of snacks including biscuits that can maintain the traditional nutritional aspects of food and show additional health benefits **(Vujić, Vitali Čepo and Vedrina Dragojević, 2015; Briliannita and Mustika Matto, 2020)**.

Scientific hypothesis

Hypothesis: biscuits with the addition of ants nest tuber and Belitung taro are significantly different in the sensory properties, nutritional value, and antioxidant activity of a variety of biscuits compared to biscuits without addition.

MATERIAL AND METHODOLOGY

Main Material

The main material used in this study was Belitung taro (*Xanthosoma sagittifolium*) and ant nest tubers (*Hydnophytum formicarum*) from the distributor in Sorong City in West Papua, Indonesia (Figure 1 and Figure 2).

Another material was tropicana slim sugar, salt, Max Crimer, Blue Bland Cookies in Mega Mall, The West Papua Indonesia.

Chemical material used in this study was (1) nutritional analysis: protein katalis, H_2SO_4, aquades, borat, BCG asam-MR, NaOH, Na2S2O3, HCl 0.02 N, petroleum ether, buffer HCl-(KC1) bisa pH 4.5, asam acetate pH 1, methanol-PA (2): DPPH analysis: 0.5 mM, methanol, dan vitamin C, vitamin E in Chemix Pratama Yogyakarta.

Chemical analyses of the Biscuits

The chemical composition of fresh biscuits (as prepared on the day of baking) was determined according to the methods of the Association of Official Analytical Chemists International **(AOAC, 2006)**: the total protein content by the Kjeldahl procedure with nitrogen to a protein conversion factor of 5.7; fat content by the Soxhlet method and ash content by carbonization. The total carbohydrate content was calculated by subtracting the sum of moisture, protein, fat, and ash percentages from 100%.

Preparation of dough and baking of the biscuits

The preparation of dough and baking of the biscuits was based on the study of **Krystyjan et al. (2015)**. Dry ingredients were mixed and then combined with others according to the recipe presented in Table 1. The dough was mixed for 10 min to obtain a homogeneous consistency and then placed into the fridge at ±6 °C for 30 min. The dough was then rolled out and 5 mm thick biscuits with a 60 mm diameter were formed and baked at 200 °C for 12 min. (Figure 3). The biscuits with the ant nest bulb powder addition in amounts of 6% and 7.5% concerning the wheat flour were prepared in the same way as the control sample (biscuits without ant nest tuber powder). The results of all the experiments are given as the average of replicates. Each replicate (biscuit) was obtained from separately prepared batches of dough.

Analysis of antioxidant activity (DPPH Method)

Antioxidant activity by DPPH method was based on **(Sompong et al., 2011; Briliannita, 2017** and **Hetharia et al., 2020)** with modification. DPPH 0.1 M was prepared by diluted 0.0039 g DPPH with ethanol 96% until 100 mL. DPPH solution (1 mL) was mixed with 200 μL aliquot and the absorbance was measured at 515 nm after 30 min incubation. The data was served as mg of vitamin C equivalent (VCE) per L with the help of the vitamin C standard curve (0 – 40 mg.L^{-1}).

Sensory analysis of the biscuits

For sensory analyses, the laboratory was equipped with special boxes and fulfilled all the basic requirements stated by SNI 01-2346-2006 (Indonesia National Standard, Organoleptic or sensory testing instructions) and sensory analysis was carried out according to **(BSN, 2006; Pauline et al., 2017)** with modification. The evaluation was performed by an untrained panel of 27 women and 3 men that were between the ages of 18 and 25 from students of the Nutrition Department Of the Polytechnic Of Ministry Of Health Of Sorong. For each sample, and for the same panelist, four repetitions were done. The evaluation was done on a Five-point hedonic scale. The scale and categories were as follows: good = 5, fair = 3 and poor = 1. Evaluated characteristics were color, aroma, taste, texture, and overall acceptance.

Statistical analysis

This study used Statistical Product dan Service Solution (SPSS) version 15.0 (Stat Soft, USA). Results were expressed by average ± standard deviation. The effect of the results was performed using the Kruskal-Wallis test (α = 0.05). The samples of individual varieties were evaluated to each other. Furthermore, all samples of individual varieties of biscuits were evaluated against common biscuit (C001).

RESULTS AND DISCUSSION

Chemical composition of the biscuits

Table 2 shows the content of essential nutrients in biscuits. After analyzing the data obtained it was found that the addition of ant nest tuber powder did not affect the carbohydrates content in biscuits – the amount of which oscillated around 60.55%. There was, however, a small but statistically significant increase in ash and water content and a significant decrease in protein and fat content in those biscuits that had additional ant nest tuber powder at 6% and 7.5%. Such a decrease can come from the fact that the ant nest tuber powder contains three times more protein and four times more ash than the Belitung taro flour that was used for baking.

Significant increase of water content for this study, because one to two water populations are identified for Belitung taro flour and are highly dependent on the temperature and concentration rather than the, showed higher retrogradation tendency. An increase in relaxation times at 75 – 80 °C is observed for flours and starches corresponding well to the amylograph pasting temperatures revealed using the Rapid Visco Analyzer **(Boakye et al., 2019)**. The results of other research show that *Xanthosoma sagittifolium* powder proportion, mixing time, and water addition, significantly affect water holding capacity, oil holding capacity, texture, and protein solubility of TVP (p <0.05). The effect of XSP addition on water holding capacity increase when added to 20% **(Lindriati, Herlina, and Arbiantara, 2018)**.

Table 1 Components used for the preparation of biscuit dough (35.25 g).

Material	C001	C013	C014
Wheat Flour (g)	17.6	17.6	17.6
Taro flour (g)	13.2	13.2	13.2
Ant nests tuber (g)	0	1.06	2.64
Vegetable Crimer (g)	0.39	0.39	0.39
Chiken eggs (g)	1.67	1.67	1.67
Margarine (g)	1.49	1.49	1.49
Baking Powder (g)	0.04	0.04	0.04
Sugar of *Tropica Slim* (g)	0.84	0.84	0.84
salt (g)	0.02	0.02	0.02

Note: C001 – without ant nest tuber powder (0%), C013 – 6% ant nest tuber powder, C014 – 7.5% ant nest tuber powder.

Table 2 Content of nutrient raw, and biscuits.

Nutrient Composition	Belitung Taro Raw	Ant Nest Tubers Raw	C001 without ant nest tuber powder	C013 with 6% ant nest tuber powder	C014 with 7.5% ant nest tuber powder
Water (%wb)	8.18 ±0.00^{a}	13.35 ±0.38^{b}	7.35 ±0.12^{a}	9.24 ±0.07^{c}	9.80 ±0.12^{c}
Ash (%db)	14.18 ±0.11^{a}	4.35 ±0.28^{b}	2.82 ±0.21^{c}	5.36 ±0.16^{d}	6.15 ±0.19^{d}
Protein (%db)	2.91 ±0.02^{a}	0.93 ±0.014^{b}	6.00 ±0.03^{c}	4.13 ±0.06^{d}	4.25 ±0.05^{d}
Lemak (%db)	0.67 ±0.05^{a}	0.62 ±0.15^{a}	23.27 ±0.06^{b}	22.17 ±0.19^{b}	19.03 ±0.007^{b}
carbohydrates (bdf*)	74.05 ±0.08^{a}	80.74 ±0.06^{b}	60.55 ±0.31^{c}	59.08 ±0.16^{c}	60.75 ±0.24^{c}

Note: Parameters in columns denoted with the same letters do not differ statistically at the level of confidence α = 0.05. Number of replications n = 4.

Table 3 Sensory evaluation results.

Characteristic	Biscuits		
	C001 without ant nest tuber powder	C013 with 6% ant nest tuber powder	C014 with 7.5% ant nest tuber powder
Colour	3.50 ±0.90^{a}	3.57 ±0.72^{b}	3.26 ±0.83^{a}
Aroma	3.86 ±0.77^{a}	3.60 ±0.96^{a}	3.30±0.95^{b}
Taste	3.90 ±0.71^{a}	4.20 ±0.96^{a}	3.30 ±1.11^{b}
Textur	3.57 ±0.77^{a}	3.83 ±0.74^{b}	3.33 ±0.92^{a}
Overall	3.80 ±0.71^{a}	4.10 ±0.72^{b}	3.53 ±0.82^{a}

Note: (a, b) The values with the same letters in the same row are not significantly different (p <0.05).

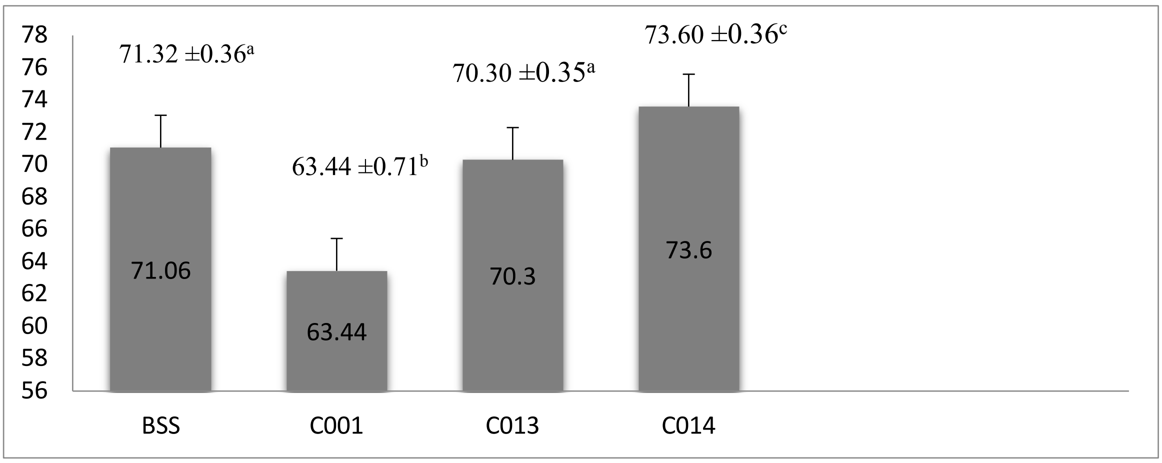

Figure 4 Ability to inhibit free radical by ant nest tuber powder (%) (DPPH Assay).

Based on the research of physicochemical properties of starches of five cocoyam cultivars where starch granule sizes varied significantly in length and width, while amylose content ranged from 11.55% (NCe002) to 33.77% (NXs001). Also, water absorption capacity (21 – 36%), pH (4.8 – 5.3), gelling point (60.5 – 69.5 °C), foam capacity (4.46 – 18.28%), bulk density (0.14 – 1.15 g.mL^{-1}) and swelling power (2.31 – 10.09) varied significantly (p <0.05) among the cultivars. Water content on our research increased **(Falade and Okafor, 2013)**. A significant increase of ash content was not caused by an interaction between Belitung taro flour with ant nest tuber powder. Product ash levels experienced an increase with increasing portions of flour (Belitung taro flour and ant nest tuber powder). This could be because of the high ash content in both materials. Another factor allegedly due to immersion with salt in the preliminary treatment Belitung taro flour. Salt contains minerals including sodium, chloride, and iodine that can be calculated in measuring ash content. Such as in the results of others our research showed that the different sections of *Xanthosoma sagittifolium* cormels were significantly different (p <0.05) in chemical composition. The apical section of all the species had high protein content while the distal section had high levels of ash, fibre, and minerals. Potassium was the most abundant mineral (763 – 1451 µg.100 g^{-1}) with appreciable amounts noted for zinc (17 – 51.1 µg.100 g^{-1}), magnesium (46.7 – 85.0 µg.100 g^{-1}), and phosphorus (41.6 – 63.1 µg.100 g^{-1}) **(Sefa-Dedeh and Kofi Agyir-Sackey, 2004)**.

Antioxidant activity

DPPH Assay

The highest antioxidant activity determined by the DPPH method was biscuit C014 (with 7.5% ant nest tuber powder) 73.60 ±0.36,(p <0.05). The lowest antioxidant activity was determined in biscuit C001 (without ant nest tuber powder) 63.44 ±0.71, p <0.05). Biscuits showed statistically significant differences p <0.05, except sample BSS and biscuit C013, due to their content of ant nest tuber powder difference (Figure 4). Significantly, the ethyl acetate extract was also the most potent antioxidant, showing 83.31% of radical scavenging activity with IC$_{50}$ 8.40 µg.mL^{-1} in the DPPH assay. The other extracts display weak to moderate antioxidative activities, ranging from 28.60 – 56.80% of radical scavenging, α-tocopherol, a positive control, shows antioxidative activity with IC$_{50}$ 6.67 µg.mL^{-1} **(Prachayasittikul et al., 2008)**. Antioxidant activity values ranged from 63.44 ±0.71 to 73.60 ±0.36 in four samples. The other research showed that extracts *Hydnophytum formicarum* could increase lymphocyte proliferation by increasing concentration. There was no chemical content difference observed on extracts *Hydnophytum formicarum*. These extracts contained flavonoids, phenolic, aldehyde/ketone, terpenoids, and tannin **(Darwis, Hertiani and Samito, 2014)**. *Hydnophytum formicarum* showed oxidant activity with IC50 = 7.03 µg.mL^{-1}. *Hydnophytum papuanum* showed oxidant activity with IC50 = 6.19 µg.mL^{-1} **(Makaba, 2017)**.

Sensory evaluation analysis

The sensory evaluation analysis is shown in Table 3. According to the data, biscuits supplemented with 6% ant nest tuber powder obtained a higher total score (4.10 ±0.82) in the sample C013 and the same as in the sample C001 (total score 3.80 ±0.71). The overall assessment allowed the classification of the product to be acceptable (3.53 ±0.82) in the case of the maximum additive (sample C014). When comparing particular sensory characteristics of four biscuits directly, significant differences (p <0.05) were observed between the sample C001 and the sample C014. On this basis, it can be concluded that the applied fortification of biscuits with ant nest tuber powder was possible and gave the desired results, at a level of 6%. Slight differences were noticed between the sample C001 and the sample C014 with a 0% and 7.5% supplementation of ant nest tuber powder. In particular, such characteristics as taste, color, aroma, and texture deteriorated. One of the main factors, as regards the deterioration of the taste of biscuits, was ant nest tuber powder; when used in significant quantities, the biscuits were leaving a crumb biscuit on the tongue. Besides, the color of the product depends on the chemical composition of ant nest tuber powder, which in turn depends on the plant species and environmental conditions **(Agatonovic-Kustrin et al., 2018)**. Thus, the flavonoid, vitamin E, phenolic, aldehyde/ketone, terpenoids, and tannins as well as the reducing sugars introduced to the product with ant nest tuber powder affected its color. Curiously, the darkening of color caused by the addition of ant nest tuber powder was not observed by the assessors.

CONCLUSION

The addition of ant nest tuber powder (7.5%) in the biscuit had an antioxidant activity (DPPH) of 73.60 ±0.36%. According to the sensory evaluation of the biscuits, they were acceptable in characteristics of the color, aroma, taste, texture, and overall. The obtained results have shown that it was statistically significant that panelists will be likely to accept the produced biscuit (p <0.05). The higher the addition of ant nest tuber powder, the higher the antioxidant activity in biscuits and lower the biscuit protein content (p <0.05).

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Contact address:

*Anjar Briliannita, The Ministry of Health of Indonesia, Department of Nutrition, Sorong, Health Polytechnic of Basuki Rahmat, KM 11, 98417, Sorong, West Papua, Indonesia, Tel. 082198200280,
E-mail: abriliannita@gmail.com
ORCID: https://orcid.org/0000-0002-6305-1222

La Supu, The Ministry of Health of Indonesia, Department of Nutrition, Sorong Health Polytechnic of Basuki Rahmat, KM 11, 98417, Sorong, West Papua, Indonesia, Tel. 0951324309,
E-mail: jurusan.gizi.poltekkessorong@gmail.com
ORCID: https://orcid.org/0000-0003-0288-9774

Corresponding author: *