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Characteristics of cured egg yolk cookies subtituted with skim milk powder during storage

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ABSTRACT: Cured egg yolk cookies is one of the processed egg products containing egg yolk which has been curing with salt and sugar. The purpose of this study was to evaluation of physical, chemical, microbiological and sensory quality of cured egg yolk cookies with various levels of skim milk powder substitution during storage at room temperature. This study consisted of the treatment of cured egg yolk substitution with skim milk powder (0; 20; 40%) and storage treatment (0; 3; 6; 9 weeks). Each treatment was replicated three times. The data of cookie quality were statistically analyzed using ANOVA. The results showed that cured egg yolk cookies with various skim milk powder substitution have similar physical and chemical characteristics, but substituted cookies had a lower fat content ($p<0.05$). There were no changes in the moisture content of cookies during 9 weeks of storage. Skim milk powder substitution could increase the peroxide number, but there was no change in the peroxide number during 3 to 9 weeks of storage. The texture of cured egg yolk cookies with 40% skim milk substitution was harder ($p<0.05$) than the 20% skim milk substitution, whereas there was no change in the texture value of cured egg yolk cookies before and after being stored for 9 weeks. There was no significant deferences in the sensory quality of cookies between unsubstituted and 40% skim milk powder substitution. In conclusion, skim milk powder substitution did not change the physical, chemical, and sensory characteristics, but could reduce fat content, increase the peroxide number and the hardness of cookies. During 9 weeks of storage, cured egg yolk cookies are still acceptable microbiologically.

Keywords: Cookies quality; Cured egg yolk; Skim milk powder; Storage

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INTRODUCTION

Cookies are baked snacks that have a sweet taste and are generally made from wheat flour, sugar, milk, and eggs which are liked by children and adults so that production and the market are increasing at this time (Putra and Suparthana, 2020). According to the Indonesian National Standard (SNI) 01-2973-1992, cookies are a type of biscuit made from soft dough, high in fat content, relatively crunchy when broken and the cross section of the piece is densely textured (BSN, 1992). To increase consumer preferences for cookies, it is necessary to diversify cookie products so that consumers do not get bored.

Cured egg yolk is a new gastronomic preparation, with the use of a mixture of salt and sugar which will gradually diffuse into the egg yolk, increasing its solidification from the outside to the inside so as to make the fat and flavor very concentrated. After solidification, egg yolks are thermally processed in an oven and this preparation process is known as yolk curing. Quickly cured egg yolks can add complex savory flavors to a variety of foods, such as salads, soups, pastas, and even meats. As a new gastronomic preparation, the process parameters for cured egg yolk have not been fully standardized. For example, the ratio of egg yolk and salt-sugar mixture, curing time in the refrigerator and post-curing heat treatment varies depending on the recipe (Lopes *et al.*, 2020). Wang (2017), has directly cured duck egg yolks using a mixture of salt and maltodextrin

Recently, food consumption is not only to satisfy hunger and to provide necessary nutrients, but also to prevent nutrition-related diseases and improve the physical and mental health of consumers. Eggs are cheap and highly nutritious food, containing 18 vitamins and minerals whose composition can be influenced by several factors such as chicken feed, age, strain, and environmental factors. Eggs are a source of protein, fat and micronutrients that play an important role in basic nutrition. However, eggs have traditionally been associated with negative factors for human health, due to their high cholesterol content (Miranda *et al*., 2015).

Eggs are very attractive from a functionality point of view, as they are a moderate source of calories (about 150 kcal/100 g), excellent in protein quality, culinary versatility and low economic cost (Carrillo *et al*., 2012), so make eggs easily accessible to the public. Eggs are also relatively rich in fat-soluble compounds and therefore can be a nutritious food for people of all ages at various stages of life. In particular, eggs may play a very important role for individuals who are at risk of undergoing low nutritional intake such as the elderly, pregnant women and children (Natoli *et al*., 2007).

Actually, eggs are a balanced food, although they have a high cholesterol content, the content of unsaturated fatty acids is higher than saturated fatty acids (Miranda *et al*., 2015). For consumers who have health problems, such as high blood cholesterol, heart disease and stroke, generally avoid consumption of high-fat foods, although it is known that the response of cholesterol in human serum to cholesterol consumption actually depends on several factors, such as ethnicity, genetic, hormonal, and the nutritional status of consumers (Miranda *et al.*, 2015). Therefore, it is necessary to have alternative cookie products to replace partially cured egg yolks so that they can provide choices for consumers who want low-cholesterol or low-fat cookies. Skimmed milk is a protein source food that has been taken from the fat, it is very suitable to be used as a partial substitute for cured egg yolks to reduce fat and cholesterol levels in cookie products.

Cured egg yolk used in the formulation of cookies is expected to provide a new taste sensation that is different from fresh egg yolk or egg yolk powder that is commonly used so far. The function of egg yolks used in food formulations as an emulsifier, because the

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proteins and lecithin present in egg yolks contribute to a very good emulsification process by stabilizing liquid suspensions with one another (Ndife *et al.*, 2010). In addition, egg yolk also has the property of absorbing oil and water so that it can help retain water and oil during the baking and storage process (Ndife *et al.*, 2010), and as a coloring agent for example in cakes and ice cream (Bhandari *et al*., 2013).

Milk-based powder is not only used for recombination or reconstitution, but also may also be exploited due to its intrinsic functional properties which can be applied as a food ingredient in several "value added foods" such as confectionery, bread, and meat products. Milk in powder form facilitates transportation, handling, processing, and product formulation applications. Milk powder has physical and functional properties including powder structure, particle size distribution, powder density, bulk density, particle density, flowability, rehydration (wetness, sinkability, dispersibility, solubility), hygroscopicity, heat stability, emulsifying properties, water activity, stickiness, and clumping (Sharma *et al*., 2012). Skim milk powder in bread formulation serves to increase the nutritional value and maintain the quality of the bread. The presence of skim milk powder may produce bread with open grains and a harder texture. In addition, skim milk powder can also increase dough stability and reduce water absorption, dough expansion and gaseous release properties of flour (Kaur *et al*., 2002).

Previous studies on the effect of various powdered milk products (skimmed milk powder, butter milk, sodium caseinate, yogurt powder, milk powder and colostrum) on the quality of cookies has been carried out by Sert *et al*. (2015). Furthermore, the application of cured egg yolks has also been studied in the manufacture of ice cream for whipping cream substitution (Agustin *et al.*, 2021). However, the characteristics of cookies containing cured egg yolk with skim milk powder substitution have not been reported so far. Therefore, the purpose of this study was to evaluate the quality of cookies which included physical, chemical, microbiological, and sensory qualities of cured egg yolk cookies with skim milk powder substitution during storage. This research is expected to provide information for the food industry to develop cookie products with cured egg yolk as an ingredient.

MATERIALS AND METHODS

This study is an experimental research consisting of treatment of substituted cured egg yolk with skim milk powder (0; 20; 40%) and storage treatment (0; 3; 6; 9 weeks) on cookies at room temperature. Each treatment was replicated three times.

Cookie ingredients

The ingredients used to prepare cured egg yolk cookies were 36 g of cured egg yolk powder, 100 g of margarine (melted), 75 g of powdered sugar, 170 g of wheat flour, 30 g of full cream milk powder, and skim milk powder. The substitution of cured egg yolk with skim milk powder was 0 (control); 20 and 40%.

Cured egg yolk powder preparation

Whole eggs were washed, the yolks were separated from the egg whites manually, then the yolks were immersed in a closed container containing a mixture of salt and powdered sugar in a ratio of 1:1 for 4 days. The egg yolks were removed and steamed for 10 minutes, then grated into smaller particles and then dried in an oven at 50^{o}C for 20 hours, then blended (Ndife et al., 2010 and Wang, 2017).

Cured egg yolk cookies preparation

Wheat flour was mixed well with full cream milk powder in a bowl. The margarine and sugar were mixed with a mixer on high speed for 3 minutes until they were pale in color and the texture was light and smooth. Cured egg yolks were added to the mixture and mixed on medium speed for 30 seconds. Next, the mixture was added half of the flour mixture / milk powder and stirred at slow speed for 1 minute. Furthermore, the remaining mixture of flour / milk powder was mixed and stirred until it

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forms a soft dough. The dough was formed into small balls (about 8g) using a mold, then baked at 150^{o} for 15 minutes. Cookies were removed from the oven and cooled to room temperature then packed in plastic and stored in a closed container for 9 weeks.

Cookie quality analysis

The cookies quality analysis includes physical, chemical, microbiological and sensory analysis. Physical analysis of cookies includes cookie diameter, thickness, spread ratio, and texture. Chemical analysis of cookies includes analysis of proximate, cholesterol and peroxide values. Sensory analysis includes color, aroma, taste, texture and acceptability, while microbiological analysis includes total plate count (TPC), total yeast and total fungus.

Cookie diameter was measured by placing 6 cookies end to end to get the average diameter in millimeters. Cookie thickness was measured by stacking 6 cookies on top of each other to obtain an average thickness (Seevaratnam *et al*., 2012). The diameter divided by the thickness gives the spread ratio. The treatment spread ratio divided by the control spread ratio multiplied by 100% is the spread factor (Kulthe *et al*., 2017). The bulk density is determined and expressed as g/cm3 (Seevaratnam *et al*., 2012). Texture analysis was carried out at week 0 and week 9. Measurement of cookie texture was carried out using a Brookfield CT3 Texture Analyzer, No. M08-372-E0315 (Brookfield Engineering Laboratories, Inc., USA) at 27°C. The standard test uses compression probe type TA44 cylinder 4 mm D, pre-load 0.1 N, pre-load speed 300 mm/minute and test speed 10 mm/minute.

The nutritional value of cookies was determined by proximate analysis according to the AOAC method (1995) which included moisture content, crude protein, fat, ash, and carbohydrates. Moisture content was analyzed by oven drying method, fat content by Soxhlet method, protein content by Kjeldahl method, ash content by dry ashing method and carbohydrates by different method. Cholesterol analysis of cookies used the Liebermann–Burchard method (Xiong *et al*., 2007).

Peroxide number is an indicator of the development of rancidity during storage, analyzed according to Sadasivam and Manickam (2008), carried out at 0; 3; 6 and 9.weeks of storage.

Sensory analysis of cookies was performed according to Giwa and Ibrahim (2012). Ten panelists participated in this study, each panelist evaluates the sample for appearance/colour, smell/aroma, overall taste/mouth feel and overall liking on a 5-point hedonic scale (1- dislike extremely, 2-dislike, 3- neutral, 4-like, 5-like extremely).

Microbiological characteristics were determined by counting the total number of microbial in cookies including total plate count (TPC), total yeast, and fungi at week of storage of 0; 3; 6 and 9. Microbiological analysis of these cookies was carried out by weighing the sample of cookies 1 g, diluted in 9 mL of physiological NaCl, and the procedure was continued to obtain a final dilution of 10^{-6}. Solution of 0.1 mL of the 10^{-5} and 10^{-6} dilutions (the corresponding decimal dilutions) were spread into each sterile Petri dish containing plate count agar (PCA) medium (Merck) for TPC and incubated at 37°C for 24 hours. For the total yeast and fungi, malt extract agar (MEA)(Oxoid) medium containing 100 ppm chloramphenicol was used with incubation time of 48 hours. The number of microbial colonies formed was counted and expressed in log CFU/g (Chen *et al*., 1983; Rostita *et al*., 2011; Eissa *et al.*, 2011).

Statistical analysis

Proximate analysis data of cured egg yolk cookies with skim milk powder substitution, physical and sensory analysis were statistically analyzed using One Way ANOVA design. Peroxide number data during storage of cookies were analyzed by Two Way ANOVA, while total bacteria (TPC), yeast and fungi were expressed descriptively. The ANOVA was performed using SPSS version 17 with a significance of $p < 0.05$.

RESULT AND DISCUSSION

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Physical characteristics of cured egg yolk cookies

Table 1 shows that the cookie diameter values for all treatments with skimmed milk powder substitution on cured egg yolks showed no difference. This may be due to the fact that cured egg yolk powder and skim milk powder both contain quite high protein content with not much different levels. Leghorn chicken egg yolk powder with 2.91% moisture content contains 39.17% protein and 53.31% fat (Wei *et al.*, 2019), while skim milk powder with 4% moisture content contains 35% protein and 1% fat (Isleten and Karagul-Yuceer, 2008). This high protein content makes the texture of cookies dense and difficult to expand when baked in the oven, so the diameters of the two types of cookies were no different. The results of this study were certainly different from the results of previous studies that compared the effect of various powdered milk products on the quality of cookies which showed that the diameter of cookies with the addition of skim milk powder, butter milk powder, yogurt powder and milk powder was higher than the diameter of cookies with sodium caseinate and powdered colostrum treatment (Sert *et al*., 2015). It is understandable that sodium caseinate and colostrum powder contain higher protein than skim milk powder, butter milk powder, yogurt powder and milk powder. Thus, the diameter of cookies is larger in cookies with lower protein content. Bovine colostrum powder contains 60-70% protein (Bodammer *et al*., 2013), while sodium caseinate (NaCas) contains 97% protein (Lin *et al.*, 2017).

The spread ratio of cured egg yolk cookies with differences in the substitution of skim milk powder showed no significant difference with an average of 3.71. These results were not much different from previous studies that the spread ratio of flour cookies with pearl millet flour (PMF) substitution first increased from 3.71 to 5.05 and then decreased significantly from 5.05 to 3.44 with increasing levels of PMF (Kulthe *et al*., 2017). The results of another study conducted by Sert *et al.* (2015), the lowest of spread ratio was found in cake samples with the addition of skim milk powder. However, the results of this study on cured egg yolk cookies with variations of skimmed milk powder to replace cured egg yolks showed no significant difference, because between egg yolk powder and skim milk powder had protein content with levels that were not too much different. This will also affect the density of cookies which showed that there was no significant difference between treatments, after the baking process. According to Hwang and Yakawa (2006), the density of cookies is also closely related to the temperature and moisture content during the baking process.

Table 1. Physical characteristics of cured egg yolk cookies with skim milk powder substitution

Skim substitution (%)	Physical characteristic of cookies (ns)					
	Weight (g)	Thickness (mm)	Diameter	Spread ratio	Spread factor (%)	Bulk density g/cm^3
0	7.42±0.10	8.83±0.30	33.30±0.37	3.74±0.17	100.00±0.00	0.97±0.05
20	7.65±0.60	8.96±0.15	33.03±0.30	3.68±0.07	98.39±1.87	0.99±0.60
40	7.43±0.08	8.86±0.23	33.10±0.65	3.73±0.17	99.82±4.60	0.97±0.33
Average	7.50±0.32	8.88±0.21	33.30±0.40	3.71±0.13	99.40±2.60	0.98±0.36

ns : non-significant

There was no difference in the spread factor of cured egg yolk cookies among several powdered skimmed milk substitutes (Table 1), with an average of 99.40%. The spread factor of this study was different from the study of Kulthe *et al.* (2017) on flour cookies with PMF substitution, starting from 136.50% and it decreased to 92.97% with increasing PMF levels.

The difference in spread factors may be caused by differences in the substitution of cookies and the components that of

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cookies. According to Kulthe *et al*.(2017), cookies containing 10% PMF with low sugar and fiber content resulted in an increase in diameter and decreased cookie thickness during baking. The appearance of cured egg yolk cookies with skim milk powder substitution can be seen in Figure 1.

a. Skim milk substitution of 0% b. Skim milk substitution of 20% c. Skim milk substitution of 40%

Figure 1. The appearance of cured egg yolk cookies with skim milk powder substitution

Chemical characteristics of cured egg yolk cookies

Moisture, protein, ash, carbohydrate and cholesterol content of cured egg yolk cookies substituted with skim milk powder with level 0; 20 and 40% showed a non-significant difference (Table 2). However, the substitution of skim milk powder can reduce the fat content of cookies ($p<0.05$). Previous studies showed that katuk leaf cookies with the addition of a mixture of skim milk and fat had a moisture content of 2.32%, ash 2.33%, protein 9.54%, fat 26%, and carbohydrates 54.96% (Putri *et al.*, 2017).

Other studies showed that cookies with the substitution of wheat flour with 50% tannia flour also had a low ash content of 1.19%, while the moisture content was 5.2%, protein 5.94%, fat 24.52%, and carbohydrates 63.14% (Putra and Suparthana, 2020). The high ash content of the cookies in this study was possible due to these cured egg yolk cookies contain a lot of salt from the egg yolk curing process with salt and sugar.

Tabel 2. Chemical characteristics of cured egg yolk cookies with skim milk powder substitution

Skim substitution (%)	Chemical characteristics of cookies					
	Moisture (%) ^{ns}	Protein (%) ^{ns}	Fat (%)	Ash (%) ^{ns}	Carbohydrate (%) ^{ns}	Cholesterol (mg/g) ^{ns}
0	7.01±0.59	8.38±0.70	25.47±1.92^{a}	14.47±1.51	44.64±2.36	1.36±0.30
20	8.60±1.15	8.26±0.16	22.54±0.68^{b}	16.79±1.11	43.78±2.45	1.15±0.12
40	7.06±1.82	8.34±0.40	21.81±0.99^{b}	15.25±1.61	47.52±2.00	1.04±0.08
Average	7.56±1.36	8.33±0.41		15.50±1.60	45.31±2.60	1.18±0.22

a; b Different letters within the same column (a, b) indicate a significant difference ($p<0.05$)
ns : not significant

This was reported by Kaewmanee *et al*. (2009) that the moisture content in egg white and egg yolk decreased gradually with increasing salt and ash content as a result of increasing salting time.

Based on the Cookies Quality Standard (SNI 01-2973-1992) (BSN, 1992), the caloric value/100 g of cookies is a minimum of 400 kcal, a maximum moisture content of 5%, a minimum protein of 9%, a minimum of 9.5% fat, a minimum of 7% carbohydrates and ash. maximum 1.5%, and the aroma and taste are normal / not rancid. The protein of cured egg yolk cookies was close to the quality standard, while the carbohydrates and fats had met the quality standard.

Cookies without skim milk substitution had a higher fat content than cookies with skim milk substitute because

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egg yolks contain a high fat while the fat in skim milk had been removed (reduced fat). In terms of cholesterol content, there was no difference in all treatments (mean 1.18 mg/g cookies). When the weight of one cookie was 7.5 g, then the cholesterol contained was about of 8.85 mg, while the average of cholesterol level in chicken eggs was 7.65 mg / g egg yolk (Aziz *et al*., 2012).

The average of moisture content of cured egg yolk cookies in this study corresponds to the moisture content of low-fat cookies containing carbohydrates as fat substitutes, which is about of 7.20% (Chugh *et al.*, 2015). However, the moisture content of cookies in this study was higher than standard cookies according to SNI-1992. Different from the study by Suma *et al*. (2014), that cookies made with a mixture of pearl millet Kalukombu variety by heating at a temperature of 200ºC for 20 minutes in an electric oven have a lower moisture content (0.58%) than a mixture of control cookies containing wheat flour (2.57%). The moisture content of cookies in this study was higher than the moisture content of cookies study by Suma *et al*. (2014). The difference in moisture content was possible due to differences in the process, temperature and heating time as well as the cookie ingredients used.

Calorie value and Recommended Dietary Allowances (RDA) of cured egg yolk cookies

Based on the carbohydrate, protein and fat content in 100 g of cookies (Table 2), the % RDA for carbohydrates, protein and fat can be calculated. When the average weight of one cookie was 7.50 g (Table 2), then cured egg yolk cookies with a weight of 100 g (about 13 pieces), each have energy values derived from:

Carbohydrates = 45.31x4 kcal	= 181.24 kcal, with % RDA	= 45.31/300 x 100 = 15.10%
Protein = 8.33 x 4 kcal	= 33.32 kcal, with % RDA	= 8.33/60 x 100 = 13.88%
Fat = 23.27 x 9 kcal	= 209.43 kcal, with % RDA	= 23.27/62 x 100 = 37.53%

The total energy of cured egg yolk cookies = 423.99 kcal, so that the consumption of 100 g (13 pieces) of cookies was only sufficient for energy needs for the general public (2000 kcal) of 423.99/2000 x 100% = 21.19%. The calorific value of cured egg yolk cookies per 100 g in this study was 423.99 kcal. Therefore, it appropriate the quality standard according to SNI, which is a minimum of 400 kcal.

Moisture content of cookies during storage

Based on Table 3, the moisture content of cookies during 9 weeks of storage at room temperature did not change, but cookies with a 20% skim substitution showed a higher moisture content than without substitution or with 40% substitution. Therefore, the 20% skim substitution was the best substitution to bind more water. However, when the substitution was increased by 40% then it becomes comparable to cured egg yolk cookies without skim milk substitution in terms of moisture content. The moisture absorption value varies based on the different constituents. The moisture absorption capacity of whole egg powder has a value of 1.60 g; egg yolk powder 0.50 g and egg white powder 1.80 g.. The absorption properties affect the rheological, functional and quality of cake products (Ndife *et al*., 2010)

The moisture absorption properties and the oil content in eggs also help retain water during the roasting process, and subsequent storage the oil will improve the physical and sensory qualities of the product (Ndife *et al*., 2010). Kusnandar *et al*. (2010) reported that the quality of cookies may decrease because it is easy to absorb water.

Peroxide number of cured egg yolk cookies

Cured egg yolk cookies without substituted skimmed milk powder showed a lower peroxide value ($p<0.05$) than cookies with skimmed milk powder substituted (Table 4). It was possible that there were

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more antioxidant compounds in egg yolks in cookies without skim milk substitution than in cookies with skim milk substitution. According to Chung and Ferrier (2006), water-soluble phosvitin in egg yolk will be denatured at a temperature of 79.7^{o}C for 10 minutes, meanwhile according to Lu and Baker (1985) the antioxidant activity of phosvitin is not affected by the pasteurization process but can be decreased by autoclaving. Thus the lower peroxide value in cookies that were not substituted with skim milk, indicated that the antioxidant compounds in egg yolks, especially phosvitin, could reduce the occurrence of lipid peroxidation in cookies. Besides phosvitin, the natural antioxidants in egg yolks are carotenoids, lecithin, and metal-binding proteins.

When egg yolk is used in food as an ingredient, phosvitin can chelate metals and prevent food from lipid oxidation (Lu and Baker, 1985).

Tabel 3. Moisture content (%) of cured egg yolk cookies with skim milk substitution during storage

Skim substitution (%)	Storage (weeks)				Average
	0	3	6	9	
0	7.02±0.59	7.70±0.30	6.50±0.48	8.05±0.28	7.32±0.72^{a}
20	8.60±1.15	7.79±0.92	8.53±0.26	9.03±0.43	8.49±0.81^{b}
40	7.06±1.82	7.46±1.48	7.30±1.22	8.20±0.68	7.50±1.24^{a}
Averagens	7.56±1.36	7.65±0.89	7.44±1.11	8.43±0.62	7.77±1.06

Different letters within the same column (a, b) indicate a significant difference ($p<0.05$)
ns : not significant

Table 4. Peroxide value (mg ekv/1000 g) of cured egg yolk cookies with skim milk powder substitution during storage

Skim substitution (%)	Storage (weeks)				Average
	0	3	6	9	
0	4.57±0.54	1.65±0.46	2.08±0.39	2.37±0.12	2.67±1.22^{a}
20	6.23±0.30	2.63±0.57	1.78±0.30	2.34±0.50	3.25±1.86^{b}
40	5.26±0.98	2.31±0.57	2.17±0.18	2.98±0.90	3.18±1.43^{b}
Average	5.35±0.92^{a}	2.20±0.63^{b}	2.01±0.31^{b}	2.57±0.60^{b}	

Different letters within the same column or the same row (a, b) indicate a significant difference ($p<0,05$)

During 3-9 weeks of storage there was no change in the peroxide value of cured egg yolk cookies. This was consistent with previous study that biscuits stored for 60 days (8 weeks) did not develop in rancidity (Nwosu and Akubor, 2018). The peroxide value was used as an indicator of the development of rancidity during storage. High temperatures are known to accelerate the rate of oxidative rancidity and peroxide formation.

The peroxide value of fresh oil is less than 10 milliequivalent/kg. When the peroxide value is between 30 and 40 millequivalent/kg, a rancid taste will be seen (Nwosu and Akubor, 2018).

Cured egg yolk cookies texture

The texture of cured egg yolk cookies with skim milk powder substitution before and after 9 weeks of storage can be seen in Table 5.

Table 5 showed that cookies with 40% skim milk substitution have a harder texture than those without or 20% skim substitution. These results indicate that more skim substituted for cured egg yolk powder caused the texture to be harder, due to the interaction of proteins in cured egg yolk powder and other proteins in cookie dough

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with skim milk protein. The results of this study were in accordance with previous study that biscuits containing milk protein powder have a harder texture than the control, and this hardness will increase with increasing levels of milk protein powder. The increased hardness of these biscuits is due to the ability of milk proteins to form a gel, retain water, fat and other ingredients in a dense matrix, resulting in a hard biscuit. This is evidenced by microscopic images showing that biscuit dough containing milk protein powder has extensive protein nets that interact with flour proteins (Gallagher *et al.*, 2005).

Table 5. Texture (N) of cured egg yolk cookies with skim milk substitution during storage

Skim substitution (%)	Storage (weeks)		Average
	0	9	
0	14.54±10.45	17.38±8.75	12.75±8.48[a]
20	15.26±2.63	22.88±15.89	14.87±9.35[a]
40	22.54±3.84	30.51±5.90	20.57±9.25[b]
Average	17.45±6.88[a]	23.59±11.11[a]	

Different letters within the same column or the same row (a, b) indicate a significant difference ($p<0,05$)

Another study also showed an increase in the hardness of cookies from 3.76 to 15.97 N with increasing levels of barley flour in cookies. The increase in hardness is related to the dissolution of wheat protein with barley flour. The interaction of barley protein granules with wheat protein makes cookies denser, thereby increasing hardness (Kulthe et al., 2017). During storage there was no change in the texture of cured egg yolk cookies, because during storage there was no change in moisture content (Table 3). Texture is a very important characteristic that contributes significantly to the overall acceptability of a food product. It is one of the three main acceptability factors used by consumers to evaluate food, the other being appearance and taste (Bourne, 1990).

Sensory characteristics of cured egg yolk cookies

The characteristics of cured egg yolk cookies with skim milk powder substitution showed on Table 6.

Table 6. Sensory characteristics of cured egg yolk cookies with skim milk substitution during storage

Skim substitution (%)	Sensory characteristics				
	Color ns	Aroma ns	Taste	Texture	Overall acceptability
0	3.83±0.98	4.27±0.66	4.16±0.78[a]	3.83±0.70[a]	4.11±0.58[a]
20	3.83±0.98	3.72±0.89	3.44±1.09[b]	3.11±1.02[b]	3.44±0.92[b]
40	3.66±0.90	4.00±0.97	4.44±0.70[a]	4.05±0.93[a]	4.22±1.00[a]

Different letters within the same column (a, b) indicate a significant difference ($p<0,05$)
ns : not significant

Based on Table 6, cured egg yolk cookies without skim milk powder substitution had no difference in color and aroma, taste, texture and overall acceptability with cookies substituted with 40% skimmed milk powder. Thus the panelists still accept the product of cured egg yolk cookies with 40% skimmed milk powder substitute, because according to the panelists' evaluation it showed that the color, aroma, taste and texture of these cookies were no different from cookies without skim milk substitution. Different from cookies in previous studies, that the substitution of

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50% wheat flour with tannia tuber flour can reduce its sensory properties (Putra and Suparthana, 2020). According to Maina (2018), the process of receiving or rejecting a food is a consideration of its multi-dimensional properties. There are three important factors that determine the acceptability of food, namely consumer characteristics, sensory characteristics, and enjoyment of food. The sensory characteristics of food such as taste, texture, aroma, and appearance have different influences and affect the acceptability of food. Therefore, sensory attributes are considered to be the main area by which food manufacturers can successfully differentiate their products.

Microbiological characteristics of cured egg yolk cookies

Microbiological characteristics evaluated in cookies with skim milk powder substitution during storage including total bacteria, yeast and fungi, showed on Table 7.

Table 7. The average of total microbial of cured egg yolk cookies with skim milk powder substitution during storage

Storage (weeks)	Skim substitution (%)	Total microbial (Log CFU/g)		
		TPC	Yeast	Fungi
0	0	0.00	0.00	0.00
	20	0.00	0.00	0.00
	40	0.00	0.00	0.00
3	0	1.02	0.00	0.76
	20	0.86	0.00	0.33
	40	0.76	0.00	0.66
6	0	1.02	0.00	0.76
	20	0.86	0.00	0.66
	40	0,76	0.00	0.66
9	0	1.10	0.00	0.00
	20	1.30	0.00	0.00
	40	1.23	0.00	0.00

Based on Table 7, during 9 weeks of storage no yeast was found in cookies, and on day 0 (before storage) no microbial were found in cookies. Starting from the 3rd week of storage, bacteria were found until the 9th week. Fungi were found in the 3rd to 6th week, but on the -9th week there was no fungal growth.

The range of total bacteria in cookie during 9 weeks of storage in this study was 1.10 to 1.30 log CFU/g or 1.26 x 10 to 1.99 x 10 CFU/g. The total bacteria from this study were lower than the results of previous studies which showed the average total bacteria of biscuits with a moisture content of 4.9% containing 20% potato starch during storage 0; 30 and 60 days respectively 3x 10^3 CFU/g; 4.5 x 10^3 CFU/g and 6.5 x 10^3 CFU/g (Seevaratnam *et al.*, 2012). Likewise, biscuits with the addition of 25-75% banana flour had a total number of bacteria ranging from 8.0 x 10^3 to 4.0 x 10^4 CFU/g and a total yeast/mold less than 1.0 x 10 CFU/g (Hernawati *et al*., 2018).

Heating is carried out in the production of biscuits at temperatures above 100ºC, causing bacteria can not grow properly and tend to decrease. The process of processing biscuits through baking causes the moisture content of the product to decrease. Baking is included in the drying process, causing a decrease in the moisture content of the material to a certain extent which can inhibit microbial growth (Effendi, 2012). There are differences the types of spoilage microbial among food products depending on the selectivity in production, formulation, processing, packaging, storage, distribution, and handling (Rawat, 2015). According to SNI

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2973:2011 (BSN, 2011), the maximum standard of microbial contamination in biscuit products is $1x10^4$ CFU/g, and the total yeast and fungus is a maximum of $2x10^2$ CFU/g. Therefore, cured egg yolk cookies with an average moisture content of 7.77% in this study, the microbiological quality was still qualify the SNI standard for 9 weeks of storage.

CONCLUSIONS

Cured egg yolk cookies with skim milk powder substitution had similar physical and chemical characteristics, but had a lower fat content. Substitution of skim milk powder could increase the peroxide value and hardness of cookies, but did not affect the sensory properties. During 9 weeks of storage, cured egg yolk cookies were still acceptable microbiologically.

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