The Effect of Chicken Eggshell Flour Addition on the Characterictics of Cookies

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Abstract: .....According to Central Statistics Agency, chicken egg production in 2020 reached 5,044,394.99 tons. 10% of whole chicken eggs are shells that just become waste. The calcium content in eggshell is about 19.20%. Therefore, chicken eggshell flour has the potency to be used as material in food processing, one of that is cookies. Cookies is one of the bakery product made from wheat flour which tastes sweet and is very popular. The aim of this study were to determine the effect of chicken eggshell flour addition to the chemical, microbiological, and sensory characteristics of cookies. The study carried out with three attempts and two repetitions, with 5%, 8%, and 10% chicken eggshell flour addition to wheat flour to make cookie dough. Based on sensory analysis, the best attempt was cookies with 5% chicken eggshell flour in addition in wheat flour. This cookie contains 4.22% water, 6.73% protein, 25.14% fat, 2.38% ash, 61.53% total carbohydrates, and 0.72% calcium. The microbiological characteristics showed the TPC number was $4{,}42x10^3$ cfu/g and no colonies were found in the EMB test. The results of hedonic test showed that the panels slightly liked the taste, color, texture parameters, and liked aroma and overall parameters.

1 INTRODUCTION

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 has a dense texture. (BSN, 1992). Cookies are flour-based bakery product which are generally made from flour, chicken eggs, vanilla, powdered sugar, margarine, baking powder, corn starch, and instant milk powder. (Mutmainna, 2013). Cookies generally have a crunchy texture and are not easily crushed like other bakery products. Cookies are one of the snacks that people are interested in, known by many people, both children, teenagers and adults, who live in rural and urban areas (Mutmainna, 2013).

Food fortification is a common thing to do, the purpose of food fortification is to add important micronutrients such as vitamins or minerals to food, so that it can improve the nutritional quality of food (WHO, 2006). One of the food products that can be fortified is cookies. Fortificant that can be added is calcium. Calcium is one of the most important micronutrients in the body. The benefits of calcium in the body are playing a role in the process of growing bones and teeth, the process of blood clotting, and helping the function of muscles in the body including the heart muscle and the respiratory muscle system (Shita and Sulistiyani, 2010). Calcium consumption in Indonesia is still relatively low at only 254 mg/day (Ministry of Health of Indonesia, 2015) compared to the Calcium Nutrient Adequacy Rate which ranges from 1000-1200 mg/day (Yulia and Darningsih, 2009).

One source of calcium that is not utilized and only ends up as waste is chicken egg shells. One of the uses of chicken egg shells in food is to make flour. The shell that encloses a chicken egg weighs 9-12% of the total egg weight and contains 94% calcium carbonate, 1% potassium phosphate and 1% magnesium carbonate (Hadi, 2005). Another study states if one medium-sized chicken egg shell produces about a teaspoon of chicken eggshell powder will provide about 750-800 mg of calcium. (King'ori, 2011).

Therefore, chicken egg shells can be used as a good source of calcium fortificant in cookies. Beside that, the use of chicken egg shell is also expected to reduce waste that is not utilized. The aim of this study

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were to determine the effect of chicken eggshell flour addition to the chemical, microbiological, and sensory characteristics of cookies.

2 RESEARCH METHODOLOGY

The research on making cookies with the addition of eggshell flour was carried out experimentally with three attempts and two replications.

2.1 Materials and Tools

The ingredients used in making cookies with the addition of chicken egg shell flour are wheat flour, chicken egg shell flour, chicken eggs, baking powder, powdered sugar, salt, margarine, skim milk, vanilla powder, and chocochips. The materials used for proximate, calcium, and microbiological analysis were methyl red, sodium oxalate, CH_3COOH, NH_4OH, H_2SO_4, $KMnO_4$, HCl, aquades, hexane, ammonia, boric acid, HCl, sulfuric acid, NaOH, butterfield's phosphate buffered (BPB) solution, nutrient agar (NA), plate count agar (PCA), Eosin Methylene Blue (EMB), ammonium oxalate, and CaCl2.

The tools used are oven (Kirin), mixer (Phillips), woks, pans, brushes, spoons, plastic spatulas, plastic gloves, analytical balance (Excellent), furnace (Thermo Scientific), desiccator, oven (Memmert), a set of soxhlet, a set of kjeldahl, porcelain cup, Erlenmeyer flask, dropper pipette, volumetric pipette, burette, stative, refrigerator, mortar and pestle, beaker glass, measuring cup, funnel, test tube, petri dish, incubator (Memmert), micropipette (DLab), Vortex (DLab), a set of sensory test equipment such as booths, glasses, plates, and spoons.

2.2 Research Steps

The steps of this research are making of chicken eggshell flour, formulation of cookies, sensory analysis, proximate, calcium, and and microbiological analysis.

2.2.1 The Making of Chicken Eggshell Flour

The making of chicken egg shell flour was carried out based on the research of Rahmawati and Nisa (2015). The following is a diagram of making chicken egg shell flour which can be seen in Figure 1.

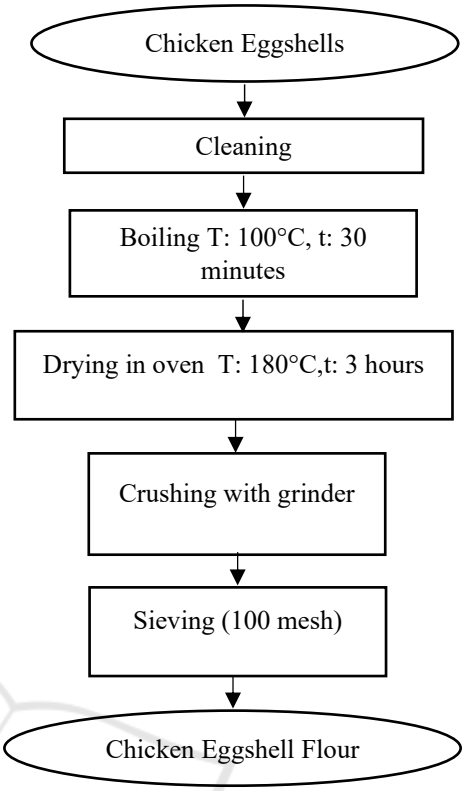

Figure 1: Flowchart of making chicken egg shell flour (Rahmawati and Nisa 2015).

2.2.2 Cookies Formulation (Dewi, 2018)

Making cookies with the addition of chicken egg shells flour using three formulations, there are adding chicken egg shell flour 10%, 8% and 5% to the weight of wheat flour. Figure of cookies can be seen in Figure 2 and the stages of making cookies can be seen in Figure 3. The three formulations of cookies can be seen in Table 1.

Figure 2: Cookies with the addition of chicken egg shell flour.

Table 1. Cookies formulation with the addition of eggshell flour.

Ingredients	F1	F2	F3
Wheat Flour : Chicken Eggshell Flour	90 grams : 10 grams (90%:10%)	92 grams : 8 grams (92%:8%)	95 grams : 5 grams (95%:5%)
Egg	A whole	A whole	A whole
Baking powde	0,25 grams	0,25 grams	0,25 grams
Powdered Sugar	35 grams	35 grams	35 grams
Margarine	55 grams	55 grams	55 grams
Skim Milk	4 grams	4 grams	4 grams
Vanilla Powder	0,25 grams	0,25 grams	0,25 grams
Chocochips	50 grams	50 grams	50 grams
Salt	0,25 grams	0,25 grams	0,25 grams

The decision of the best formulation of cookies is done through a hedonic test on panelists which includes taste and texture. The formulation preferred by the panelists was used as the main formulation and continued with sensory comparison tests with cookies on the market, proximate and calcium tests, and microbiological tests.

2.2.3 The Making of Cookies

In making the cookies, the first step is mixing margarine, powdered sugar, skim milk and chicken eggs until they are homogeneous and the color turning to pale white in. Then slowly add the flour, chicken egg shell flour, baking powder, and chocochips. Next, the dough is formed on a baking pan, and baked in an oven at 160ºC for 30 minutes. The making cookies can be seen in Figure 3.

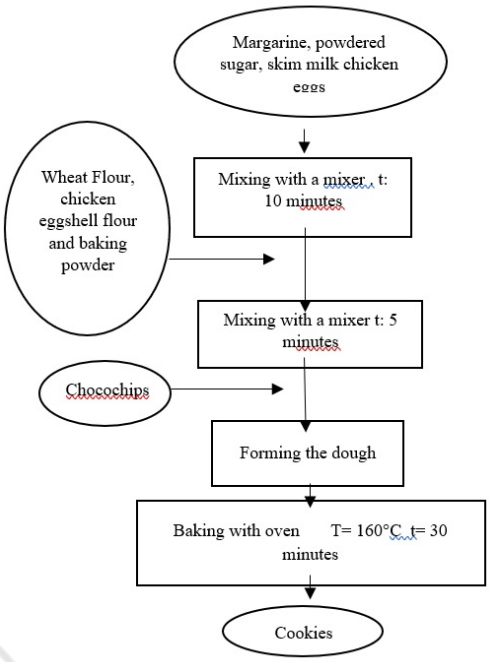

Figure 3: Flowchart of Making Chicken Eggshells Flour Cookies (Dewi, 2018).

2.2.4 Sensory Analysis

Sensory analysis in this study was carried out in two stages. The first stage was a hedonic test to determine the best formulation decision on 15 panelists with taste and texture parameters. The results of this test were further tested by Duncan with a level of 5%.

The second stage of sensory analysis to determine the level of preference of the selected formulation compared to cookies on the market. This analysis was conducted on 15 panelists with parameters of taste, aroma, color, texture and overall. The results of this test were further tested by t-test with a level of 5%.

The hedonic test assessment format is carried out according to Simanungkalit *et al*. (2018) which consists of 7 scale acceptability, score 1 = very dislike, score 2 = dislike, score 3 = slightly dislike, score 4 = neutral, score 5 = slightly like, score 6 = like, and score 7 = very like.

2.2.5 Water Content Analysis (AOAC 2007)

In determining the water content, the direct method is based on weighing the weight of the sample. The difference between the initial weight of the sample and the dry weight is the water content. The dry and clean porcelain was kept constant in the oven, followed by weighing 2 grams of the sample in the porcelain. The sample in the porcelain is dried using

an oven at 105°C for 4-6 hours until a constant weight is obtained. The formula of water content:

$$Water\ Content = \frac{weight\ of\ water\ loss\ (g)}{Sample\ weight\ (g)} \text{x}100\% \quad (1)$$

2.2.6 Ash Content Analysis (AOAC 2007)

Ash is an inorganic substance obtained as a result of burning an organic material. Ash in the material is determined by weighing the remaining minerals as a result of burning organic sample at a temperature of 550°C. The porcelain to be used must be constant, then the weighed sample is added and burn in the furnace until white ash is formed (4-6 hours), and weighed until it is constant. The formula of ash content:

$$\text{Ash Content} = \frac{Ash\ weight\ (g)}{sample\ weight\ (g)}\ x\ 100\% \quad (2)$$

2.2.7 Fat Content Analysis (AOAC 2007)

The fat or oil fraction can be separated by extracting with a fat solvent such as petroleum ether, ethyl ether, chloroform, hexane, and benzene, then evaporated and the fat content calculated.

The dry sample was weighed and then put into the hull. The sample in the hull is inserted into the Soxhlet apparatus and 50 ml of hexane is added. This analysis is carried out until all the fat in the sample is extracted. The extracted fat was then dried to remove the solvent and weighed to a constant. The formula of fat content:

$$\text{Fat Content} = \frac{Fat\ Content\ (g)}{Sample\ Weight\ (g)}\ x\ 100\% \quad (3)$$

2.2.8 Protein Content Analysis (AOAC 2007)

Protein determination is based on the oxidation of carbonaceous materials and the conversion of nitrogen into ammonia. The ammonia reacts with acid to form ammonia sulfate. The solution is made alkaline and the ammonia is evaporated and then absorbed in a solution of boric acid. The nitrogen contained in the solution can be determined by titration with 0.1 N HCl.

Samples of 0.5 grams, 2 grams of selenium mixture, and 10 ml of concentrated sulfuric acid were put into a Kjeldahl flask and then destructed in an acid chamber until a clear green color. The sample was cooled and transferred to a 100 ml volumetric flask and then adjusted to the line with distilled water. 25 ml of boric acid and 3 drops of N indicator were put into the Erlenmeyer as a distillate container. Into the distillation apparatus, 10 ml of sample and 20 ml of 30% NaOH were added. The sample was distilled until all the NH_3 was accommodated in the Erlenmeyer. The accommodated distillate is titrated with HCl 0,1 N until it turns to pink. The formula of protein content:

$$N\ Content = \frac{(Vx\ N\ HCl\ x\ DFx14{,}007)}{sample\ weight\ (mg)} x100\% \quad (4)$$

$$\text{Protein Content= N Content X Coversion Factor} \quad (5)$$

2.2.9 Carbohydrate Content Analysis (AOAC 2005)

Carbohydrates are a group of compounds consisting of the elements carbon, hydrogen, and oxygen. Carbohydrate analysis was carried out using the by difference method. The formula of carbohydrate content:

$$\text{Carbohydrate Content (\%) = 100\% – (water content + protein content + ash content + fat content)} \quad (6)$$

2.2.10 Calcium Content Analysis (Mursyidi and Rohman, 2006)

Analysis of calcium content using the permanganometry method. The principle of this method is the oxidation-reduction reaction. The standard solution used in this titration method is potassium permanganate ($KMnO_4$), $KMnO_4$ is a strong oxidizing compound. First, the sample is ashed, then the ash is dissolved with concentrated HCl, and calcium is precipitated as calcium oxalate, then the precipitate is dissolved with sulfuric acid (1:4), and titrated with potassium permanganate (0,1 N). The formula of calcium content:

$$Ca\ Content = \frac{(Vx\ N\ KMnO4\ x\ Mr\ Ca)}{Sample\ Weight\ (g)} x100\% \quad (7)$$

2.2.11 Total Plate Count Analysis (AOAC 1996)

The purpose of the Total Plate Count (TPC) analysis is to detect the presence or absence of total microbes in chicken egg shell flour cookies. The working principle of TPC analysis is the growth of microorganisms after being incubated in Nutrient Agar media at 37°C for 48 hours, then these microorganisms will grow and forming colonies that can be directly counted.

2.2.12 Analysis of Coliform Bacteria (AOAC 1996)

Coliforms are excreted from the digestive tract of animals and humans. The purpose of the coliform analysis is to determine the presence or absence of *Escherichia coli* bacteria in chicken eggshell cookie

products. Coliform analysis using Eosin Methylene Blue (EMB) agar media. This method is carried out by taking 1 ml of a suspension of cookies product that have been diluted in BPB then put into an empty petri dish and then poured EMB agar liquid media and homogenized. Then the petri dishes were incubated at 37^{o}C for 24 hours.

2.3 Hypothesis

The hypothesis of this research is cookies with the addition of chicken egg shell flour will be accepted by the panelists, beside that the chemical and microbiological qualities will close to the cookies quality that set by Indonesian National Standards.

3 RESULT AND DISCUSSION

3.1 Sensory Analysis to Determine the Best Formulation

Based on the results of the sensory test with hedonic which includes texture and taste then further tested with Duncan with a level of 5% showing the selected formula is formulation 3. The results of the hedonic test can be seen in the table. 2.

Table 2. Hedonic test results to determine the best formulation.

Parameter	F 1	F 2	F 3
Taste	1,9^{a}	2,3^{a}	4,5^{b}
Texture	1,4^{a}	2,1^{b}	4,7^{c}

Based on the results of the hedonic test analysis of the three cookie formulations and continued with the 5% Duncan test, the three cookie formulations have significant differences. The cookies that were preferred by the panelists were formulation 3 with the addition of 5% chicken egg shell flour to the wheat flour. These results are in accordance with the research of Rahmawati and Nisa (2015), cookies with the addition of chicken eggshell flour as much as 5% have the best sensory properties (Rahmawati and Nisa, 2015). The addition of 5% chicken egg shell flour to the wheat flour produces cookies with a crunchy texture and better taste. So that formulation 3 is used for further testing.

3.2 Proximate and Calcium Analysis

Formulation 3 which is the best formulation was tested for water content, ash content, fat content, protein content, carbohydrate content and calcium content. Proximate and calcium test results can be seen in Table 3.

The water content affects the quality of the cookies, if the water content is too high it will decrease the crunchiness of the cookies. This is due to the large amount of water bound to the carbohydrate matrix (Basrin *et al.,* 2016). Based on table 3, the water content of cookies with the addition of chicken egg shell flour is 4.22%. This in accordance with the requirements for the quality of cookies based on the Indonesian National Standard, which is maximum of 5% (BSN, 2011).

Table 3. Results of Proximate Analysis and Calcium Cookies Chicken Eggshell.

No.	Parameter	Results
1	Water	4,22 %
2	Ash	2,38 %
3	Fat	25,14 %
4	Protein	6,73 %
5	Calcium	0,72 %
6	Carbohydrate	61,53 %

Ash content shows the total mineral content of a product. Based on table 3, the ash content of cookies with the addition of chicken egg shell flour is 2.38%. The composition of cookies is dominated by wheat flour. The ash content of wheat flour allowed is a maximum of 0.7% (BSN, 2009).

The fat content of cookies with the addition of chicken egg shell flour is 25.14%. The high fat content in cookies is influenced by the addition of the ingredients, margarine, skim milk, eggs, and chocochips. Margarine generally has a fat content of 80-81%, skim milk 14-16% (Ulfa *et al*., 2017), and chicken eggs have a fat content of 12% (Nova, 2014).

The protein content of cookies with the addition of chicken egg shell flour is 6.73%. This is in accordance with the requirements for the quality of cookies based on the Indonesian National Standard, which is at least 5% (BSN, 2011). Widiantara *et al.* (2018) stated that the factors that affect the high protein content are the composition of the ingredients for making cookies such as skim milk, eggs, powdered sugar, margarine, flour, and others.

Carbohydrate content in cookies with the addition of chicken egg shell flour is 61.53%. This is in accordance with the requirements for the quality of cookies based on the Indonesian National Standard,

which is at least 70% (BSN, 2011). Nurdjanah et al. (2011) stated that one of the factors that affect carbohydrate content is wheat flour as the main ingredient in cookies. In addition, Fatkurahman et al. (2012), stated that the carbohydrate content calculated by difference is influenced by the content of other macronutrients such as protein, fat, water, and ash, if the other nutritional components are lower, the carbohydrate content will be higher.

Cookies with the addition of chicken eggshell flour have a calcium content of 0.72%. The Food and Drug Supervisory Agency (2018) states that products that may include claims for calcium sources must contain calcium not less than 15% ALG/100 grams (in solid form) or equal to 165 mg/100 grams. As for the high calcium claim, it contains calcium not less than 2 times the amount for the calcium source claim, which is 330 mg/100 grams, and for the general requirements for the packaging label claim, the serving size is 30 grams. In cookies with the addition of chicken eggshell flour if based on a per serving (30 grams) is 216 mg/100 grams, then these cookies are not allowed to include a "high calcium" claim.

3.3 Microbiological Analysis

Microbiological analysis on cookies with the addition of chicken eggshell flour was the total plate count and coliform bacteria. The number of bacteria contained in a product can be determined by examining the total plate count (Mursalim, 2018). The total plate count can be related to shelf life, quality and hygiene at the time of production but this is not related to the food hazard and safety of a product. (BPOM, 2012).

Based on the results of the analysis that has been carried out, the total plate count on cookies with the addition of chicken egg shell flour is 4,42 x 10^3 cfu/g. According to the requirements of Indonesian National Standard on cookies, the total plate count in cookie products should not be more than 10^4 cfu/g, so that the cookie production process has implemented good manufacturing practice.

The coliform test is an indicator to determine fecal contamination in water (Putri and Pramudya, 2018). One of the main ingredients for making cookies is water. Cookies with the addition of chicken egg shell flour showed a negative coliform test result. According to Indonesia National Standard on cookies, the maximum amount of coliform in cookies contains <20 APM/gram, so that chicken eggshell flour cookies are safe for consumption.

3.4 Hedonic Test

To find out the level of consumer preference for the selected cookie formulation, a comparison test was carried out with cookies on the market. The test results can be seen in Figure 4.

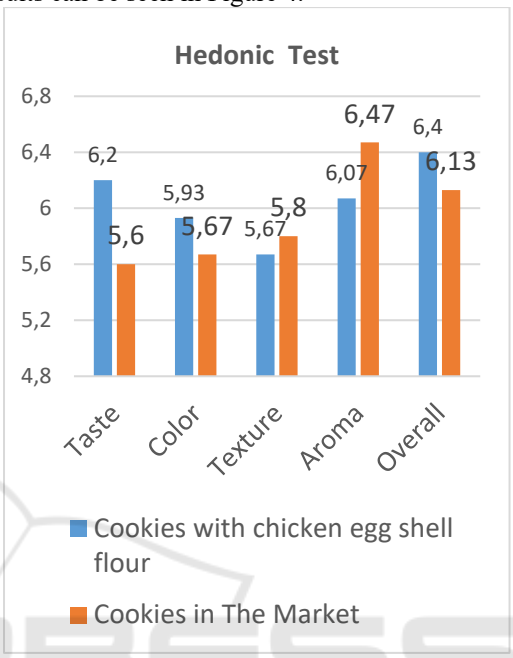

Figure 4: Results of Hedonic Test of Cookies with Addition of Chicken Egg Shell Flour and Cookies on the Market.

The results of the hedonic test followed by a t-test with a level of 5% showed that cookies with the addition of chicken eggshell flour and cookies on the market had no significant difference. However, the graph and the score results showed cookies with the addition of chicken eggshell flour are slightly more preferred in taste, color, and overall parameters than cookies on the market.

3.4.1 Taste

Taste is one of the most important factors in determining the consumer final decision to accept or reject a food (Thariq *et al*., 2014). The results of the hedonic test for taste parameters showed that the panelists liked cookies with the addition of chicken egg shell flour with an average value of 6.2 compared to cookieson the market with an average value of 5.6. Market cookies show a very sweet taste, while cookies with chicken egg shell flour are not too sweet, so the panellists were preferred that.

3.4.2 Color

Color is an important criteria for the acceptance of food products and is one of the main criteria (Rymbai et al., 2011). The results of hedonic test for color parameters showed that panelists preferred cookies with the addition of chicken egg shell flour with an average value of 5.93 than cookies on the market with an average value of 5.67. From the results of the test, it can be seen that eggshell cookies are preferred by consumers because they have a brighter color than the color of cookies on the market.

3.4.3 Texture

Texture is a feature of a material as a result of a combination of several physical properties such as size, shape, amount, and elements of the formation of materials that can be felt by the senses of touch and taste (Midayanto and Yuwono, 2014). Based on Figure 4, the panelists liked the texture of cookies on the market with an average of 5.80 compared to the texture of cookies with the addition of chicken egg shells with an average of 5.67.

Panelists do not like the texture of cookies with the addition of chicken egg shells flour because these cookies have a harder texture. According to research of Suryati et al. (2019), the addition of more chicken eggshell flour is directly proportional to the hardness of cookies. This is because chicken egg shell flour has a coarse texture and low water content of 0.996% (Syam et al., 2014).

3.4.4 Aroma

Aroma is in the form of molecules of volatile compounds that are inhaled by the sense of smell which play an important role in consumer acceptance (Bahmid et al., 2019). The results of hedonic test for aroma parameters, the panelists like cookies on the market with an average of 6.47, compared to the aroma of cookies with the addition of chicken egg shells with an average of 6.07. Panelists do not like the aroma of cookies with the addition of chicken eggshell flour because the original aroma of cookies is slightly lost due to the addition of chicken eggshell flour. According to Merta et al. (2020), chicken eggshell flour was not able to attract the panellists preference because chicken eggshell flour did not have a distinctive aroma. According to Mardini et al. (2007) the raw materials used in the manufacture of the product will affect the aroma of the final product.

3.4.5 Overall

The results of the hedonic test for the overall parameter, showed that the panelists preferred cookies with chicken egg shell flour with an average of 6.40, compared to market cookies with an average of 6.1. Overall, the panelists liked cookies with the addition of chicken egg shell flour due to the existing sensory attributes such as the taste and color preferred. According to research conducted by Suryati et al. (2019), the final product of cookies is influenced by the materials used and will affect the panelists preference for cookies.

4 CONCLUSION

Based on the hedonic test on the three formulations, it showed that the panelists preferred cookies with the addition of chicken egg shells 5% to the wheat flour. Cookies with the addition of chicken egg shell flour 5% to the wheat flour contains water 4.22%, ash 2.38%, fat 25.14% protein 6.73% calcium 0.72% carbohydrate 61.53%, total plate 4.42 x 10^3 cfu/g, and showed negative results for the coliform test. Based on the hedonic test by comparing cookies with the addition of chicken egg shells 5% to wheat flour with cookies on the market showed there is no significant difference in the parameters of color, taste, aroma, texture and overall.

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