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**Original Article**

# Enhancement of Nutritional and Organoleptic Qualities in Biscuits through Substitution with Pumpkin Puree and Katuk Leaves

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**ABSTRACT**

Biscuits, a popular snack, offer a platform for nutritional innovation by incorporating local food ingredients. This study explores the substitution of traditional ingredients with pumpkin puree and katuk leaves to develop enriched biscuits suitable for pregnant women and nursing mothers. Employing a Completely Randomized Design (CRD), the study varied the levels of pumpkin puree and katuk leaf substitutions across four formulations: F0 (0% pumpkin, 0% katuk leaves), F1 (40% pumpkin, 3% katuk leaves), F2 (45% pumpkin, 4% katuk leaves), and F3 (50% pumpkin, 5% katuk leaves). Conducted in Makassar City in 2023, the study utilized SPSS for data analysis. Results indicated that biscuits with pumpkin and katuk leaf substitutions showed enhanced antioxidant properties, evidenced by significant increases in phenolic compounds ($p=0.003$) and flavonoids ($p<0.001$). Additionally, these biscuits met the moisture and ash content standards set by SNI 2973:2011 and SNI 01-2973-1992, respectively. Organoleptically, the F1 biscuits were most preferred, scoring 783. In conclusion, substituting traditional biscuit ingredients with pumpkin puree and katuk leaves significantly improves the antioxidant activity, compliance with moisture and ash standards, and organoleptic quality of the biscuits.



## INTRODUCTION

In recent years, attention to the nutritional and organoleptic quality of food has increased, along with public awareness of the importance of a healthy diet. Biscuits are one of the most popular snack foods due to their practicality, portability, readiness, and potential to be modified to be more nutritious without compromising on flavour, making them a top choice for daily snacks. However, their nutritional content is often inadequate to fulfil people's nutritional needs. The results of a survey conducted online by *The Harris Poll* with participants aged 18 years and over, stated that 77% of Indonesians consume more snacks than heavy meals every day.[1] Biscuits as a snack are expected to contribute energy and other nutrients needed by the body to replace the energy that has been expended during activities.

Biscuits are dry bakery products made by baking dough made from wheat flour with substitutes, oil/fat, with or without the addition of other food ingredients and permitted food additives. Biscuits are made using a wheat flour base and added with other additives, such as sugar, eggs, margarine, *emulsifiers*, *shortening* and flavour ingredients. Biscuits according to SNI 2973:2011 have a moisture content of less than 5%, so that biscuits can be stored longer, protected from moisture, and become a practical food for consumers.[2]

Pumpkin *(Curcubita moschata*) belongs to the *Cucurbitaceae* family, which in fresh form is

often processed as a vegetable and wet cake ingredient. Pumpkin is a potential source of carbohydrates and vitamin A, as it contains carotenoid compounds of 767µg/g fresh fruit.[3] Pumpkin flesh contains antioxidants and increases appetite. Pumpkin fruit is a local food that contains a lot of inulin and dietary fibre needed for health maintenance, especially the digestive tract.[4]

Mona et al. stated that biscuits formulated with extracted soy protein (PKT) up to a level of 20% along with cassava flour and pumpkin flour as a substitute for wheat flour, will produce products with nutritional value, fibre and carotene levels that are much higher than control products at the same level of consumer acceptance.[5] This research provides an innovation where biscuits are substituted using pumpkin puree instead of pumpkin flour, so that the manufacturing process is easier, faster and simpler. However, in terms of nutritional quality, these biscuits are richer in nutrients.

Katuk leaf *(Sauropus androgynous*) is a fruit group vegetable plant that is widely found in Southeast Asia. This vegetable contains *sterol* compounds that are *estrogenic*, so it can spur the production and release of breast milk. This vegetable is also rich in chlorophyll which can cleanse body tissues and metabolic waste, is antiparasitic, antiviral, and cleanses toxins in the body.[6] Katuk leaves contain high protein, beta-carotene, vitamin A, vitamin B1, vitamin C, calcium, iron, and magnesium. Katuk leaves were chosen to be substituted in biscuits because the iron content of boiled katuk leaves is 3.1 mg/100g higher than boiled green spinach 0.5 mg/100g. In addition, katuk leaves are also easily obtained at a low price. Katuk leaves also contain tannins, saponins, flavonoids, and papaverine alkaloids.

The results of research by Suryati, et al. state that the best treatment is cookies with the addition of 53% pumpkin puree and 7% chicken eggshell flour can affect ash content, calcium content and texture.[7] The results of other studies also show that the addition of pumpkin flour has a significant effect on carbohydrate and protein levels in cookies.[8] Research by Sariani et al found that the addition of katuk leaf flour had a very significant effect on sago biscuit products with organoleptic tests.[9] The results of research by Retno Sri Lestari, et al, showed that the substitution of katuk leaves by 5% in cookies was acceptable to panellists with calcium levels of 120mg/100g cookies and iron levels of 0.14mg/100g cookies.[10] The results of these studies did not use the composition of pumpkin puree and katuk leaves, but pumpkin puree with chicken eggshell flour, katuk leaves were processed first into flour and no composition of pumpkin puree and katuk leaves was found to be substituted in biscuits, but more in cookie products.

The results of some of these studies have not used pumpkin in puree form and katuk leaves in biscuits. This study developed biscuits using local foods that do not require a long time by making flour first from these local foods. This study aims to assess the impact of replacing wheat flour with pumpkin puree and katuk leaves on the nutritional and sensory quality of biscuits, with the aim of developing nutritious local food-based biscuits for pregnant and lactating mothers.

## METHODS

This type of research is experimental with a Completely Randomised Design (CRD), so that each formula can be tested under the same conditions, thus minimising possible bias and randomisation in the placement of formulas. In addition, it is also in accordance with the purpose of this study, which is to assess the impact of the substitution of pumpkin puree and katuk leaves in biscuits on the nutritional and sensory quality of biscuits. The substitution factors of pumpkin puree and katuk leaves for each formula were F0 = 0%: 0%, F1 = 40%: 3%, F2 = 45%: 4% and F3 = 50%: 5%. The determination of the pumpkin puree and katuk leaf ratio formula was obtained from the results of the research team's trials conducted 8 times by considering the results of Retno Sri Lestari, et al., research, which used 5% katuk leaves. Each formula was tested for antioxidant activity, moisture content, ash content and for the treatment formula, organoleptic test was conducted.

This research was conducted from March to July 2023 at the Food Technology Laboratory and Organoleptic Laboratory of the Nutrition Department of the Makassar Health Polytechnic,

Integrated Biotechnology Laboratory of the Faculty of Animal Husbandry, UNHAS Makassar.

The materials used in this study can be seen in table 1 below:

**Table 1. Ingredients of Pumpkin Puree and Katuk Leaf Substitution Biscuits**

| Material | F0 | F1 | F2 | F3 |
|---|---|---|---|---|
| Wheat flour (g) | 125 | 100 | 110 | 120 |
| Tapioca (g) | 25 | 25 | 25 | 25 |
| Butter unsalted (g) | 30 | 30 | 30 | 30 |
| Egg yolk (g) | 8 | 8 | 8 | 8 |
| Powdered sugar (g) | 62.5 | 62.5 | 62.5 | 62.5 |
| Liquid milk (ml) | 50 | 50 | 50 | 50 |
| Pumpkin puree (g) | 0 | 60 | 67.5 | 75 |
| Katuk leaves (g) | 0 | 4.5 | 6 | 8 |
| Baking powder (g) | 2 | 2 | 2 | 2 |
| Ammonia cake (g) | 1.5 | 1.5 | 1.5 | 1.5 |
| Salt (g) | 1.5 | 1.5 | 1.5 | 1.5 |
| Vanilla (g) | 2 | 2 | 2 | 2 |

The tools used include: food scales, oven gutter, spoon, whisk, *noodle maker*, basin, oven, and spatula. The procedure for making biscuits was as follows:

1. Preparation of substitution ingredients: Steamed pumpkin that has been mashed, roasted at $70^0$C for 20 minutes. The katuk leaves were washed under running water and blanched for 2 minutes. Then pumpkin, katuk leaves, and liquid milk were mixed and blended for 3 minutes.
2. Preparation of biscuit dough: Egg yolks are beaten for 2 minutes, add powdered sugar, baking ammonia, salt and vanilla, and mix well. Add unsalted butter and tapioca. Then add the substitute ingredients, mix well. Then add wheat flour and mix until it forms a dough that can be rolled with a noodle maker.
3. Mould the biscuits into a round shape with a hole at the top.
4. Bake the biscuits for 30 minutes at $130^0$C. Then the biscuits are removed, cooled, and packaged.

**Antioxidant Activity Testing**

The antioxidant activity of the sample extract was determined by measuring the concentration of DPPH free radicals as follows: 1 ml of sample extract was added to 1 ml of DPPH solution (0.16 g/L) in methanol, then shaken. After that, the absorbance was measured after 30 minutes at room temperature at a wavelength of 517 nm and as a blank, methanol was used with the same process as mentioned above.

DPPH inhibitory activity (%) $=\frac{A0-A1}{A0}$x100%

**Water Content Testing**

Moisture content was measured using the thermogravimetric method as follows: Clean porcelain cup was ovenised at $105^0$C for 2 hours. Then cooled in an applicator for ½ hour then weighed (A g). Next, weigh ± 1 g of sample (B g) into the porcelain cup. Then heat in the oven at $105^0$C for 8 hours or leave overnight. Remove from the oven and cool in an applicator for ½ hour then weigh (C g). The calculation is.[11]

% Dry matter = $\frac{C-A}{B}$x100%

% Water = 100% - % Dry Material

**Ash Content Testing**

Ash content was measured using the thermogravimetric method as follows: Porcelain cup along with the sample is put into the electric furnace in the determination of water content. The temperature of the furnace is set to $600^0$C, then left for 3 hours until it becomes ash (to accelerate the ignition process once in a while the furnace is opened). Then let it cool down a bit and put it in an exicator for ½ hour. Then weigh (D g). The calculation is

% Ash = $\frac{D-A}{B-A}$x100%

## Organoleptic Testing

Organoleptic tests were carried out using untrained panelists, namely third-year students of the Department of Nutrition who had taken Food Technology and Food Sensory courses, so that the panelists already knew the basis for assessing food products using organoleptic tests. There were 50 panellists selected by purposive sampling. The organoleptic test was conducted to determine the most acceptable biscuit formula from the aspects of colour, aroma, texture and taste, using a hedonic scale. The assessment score consists of 5 levels, namely very much like (5), very like (4), like (3), somewhat like (2) and dislike (1).

Data for each parameter was inputted into the SPSS programme and tested for normality. If the data was normal, then One Way Anova test was conducted with 95% confidence degree and Duncan's further test. If it was not normal, it was tested by further analysed with Kruskal-Wallis test with 95% confidence degree and Mann Whitney further test.

## Ethical Clearance

This study was conducted in accordance with the ethical recommendations of the Health Research Committee (KEPK) of the Makassar Poltekkes Kemenkes No. 136 / KEPK-PTKMS / IV / 2022.

## RESULTS

**Table 2. Mean values of antioxidant activity in biscuits**

| Characteristics | Formula (Mean ± SD) | | | | p* value |
|---|---|---|---|---|---|
| | F0 | F1 | F2 | F3 | |
| Antioxidant activity | 17.9±1.0 | 20.7±0.3 | 24.6±6.2 | 39.2±2.0 | 0.010 |
| Inhibition power | 3.1±0.0 | 4.7±0.1 | 3.9±0.2 | 5.3±0.0 | 0.000 |
| Phenol | 74.1±2.0 | 70.1±0.6 | 59.0±1.6 | 52.2±4.1 | 0.003 |
| Flavonoids | 9.3±0.0 | 9.0 ±0.00 | 5.9±0.1 | 5.40±0.3 | 0.000 |

*Anova one way test

F0=biscuits with 0% pumpkin and katuk leaf substitution, F1=biscuits with 40%:3% pumpkin and katuk leaf substitution, F2=biscuits with 45%:4% pumpkin and katuk leaf substitution, F3=biscuits with 50%:5% pumpkin and katuk leaf substitution, *One-way ANOVA test.

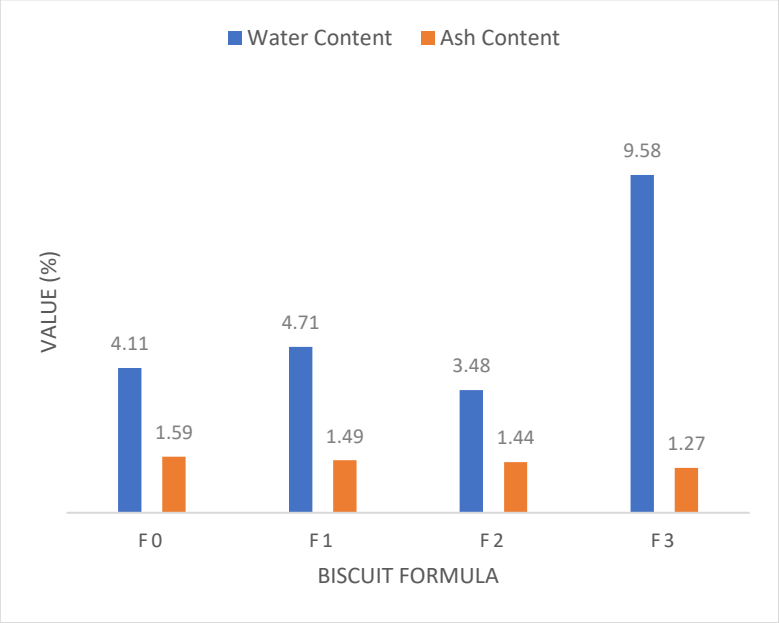


**Figure 1: Water Content and Ash Content of Biscuits**

**Table 3. Organoleptic Test Results of Pumpkin Puree and Katuk Leaf Substitution Biscuits on Untrained Panelists**

| Parameters | Mean Value of Biscuit Organoleptic Test | | | *p value |
|---|---|---|---|---|
| | F1 | F2 | F3 | |
| Colour | 4.10 | 3.94 | 3.52 | 0.002 |
| Aroma | 4.18 | 4.10 | 3.72 | 0.005 |
| Teksture | 3.38 | 3.42 | 2.96 | 0.011 |
| Flavour | 4.02 | 3.82 | 3.44 | 0.000 |

*Anova one-way test
The average judgement for each parameter (F1, F2, F3).

## DISCUSSION

The biscuits showed positive antioxidant content with an average range of 17.91 mg/100g to 39.20 mg/100g, with an average free radical inhibition of 3.14% to 5.30%. This was reinforced by the presence of phenol and flavonoid compounds in the biscuits, although the levels decreased as the substitution of pumpkin puree and katuk leaves increased. However, the results of the one-way ANOVA test stated that there was a significant effect of the substitution of pumpkin puree and katuk leaves in biscuits on the content of phenol compounds (p value = 0.003) and flavonoids (p value = 0.000). This proves that antioxidant activity is directly proportional to the content of phenol and flavonoid compounds. As the results of research by Kemit, Widarta, and Nocianitri state that antioxidant activity is influenced by the amount of flavonoid compounds contained in avocado leaf extract, the more flavonoid compounds, the more antioxidant activity increases.[12] Similarly, the results of Dainy & Yunieswati's research on spice biscuits with a milk and purple sweet potato flour ratio of 2:2 had strong antioxidant activity (14.6 µg/mg).[13]

The antioxidant activity of the biscuits in this study was influenced by the concentration of pumpkin and katuk leaves. Antioxidant content in plant-based ingredients acts as a radical catcher, and helps convert radicals into less reactive species. Various antioxidants such as phenolics and flavonoids as free radical catchers are widely found in food sources such as fruits, vegetables, tea and others.[14] Katuk leaves as a local plant have high green pigments, in which there are large amounts of antioxidants that are very efficacious to reduce free radicals in the form of flavonoid and polyphenol compounds.[15] Katuk leaves contain flavonoids that have strong antioxidant abilities, because they have antioxidant compounds with IC50 values of 50-100 ppm. A compound is said to be a very strong antioxidant if the IC50 value is less than 50 ppm, strong for IC50 values of 50-100 ppm, moderate if 100-150 ppm, and weak if the IC50 value is 151-200 ppm.[16,17,18] Meanwhile, pumpkin powder has an IC50 value of 9,900 ppm.[19,20]

The results also showed that there was no significant difference (ρ=0.083) in biscuit moisture content. However, the moisture content of biscuits substituted with pumpkin puree and katuk leaves can be said to be in accordance with the requirements of SNI 2973: 2011, which is a maximum of 5%. This situation is in accordance with the statement of Estiasih, T. and Ahmadi, K.G.S if the greater the temperature difference between the heating medium and the food material, the faster the heat transfer to the food material and the faster the evaporation of water from the food material, so that it will minimize the time used. After the heating process, water moves from high pressure to low pressure, but not all water comes out and evaporates, so the biscuits still contain low water content.[20] Likewise, ash content can affect biscuits. The results prove that the ash content of biscuits substituted with pumpkin puree and katuk leaves is in the range of 1.27%-1.59% and has met the SNI 01-2973-1992 standard, which is a maximum ash content of 1.5 g/100 g. The higher the ash content, the higher the ash content. The higher the ash content, the higher the minerals contained in the biscuits. The ash content of pumpkin puree and katuk leaf substitution biscuits decreased, along with the increase of pumpkin puree and katuk leaf substitution. The measurement of ash content aims to determine the amount of mineral content contained in a material, which can affect the color of biscuits, poor texture and not crispy. As the results of Nabilla's research, showed that the higher the proportion of mixed flour (10% moringa leaves, 20% soy flour, 20% fish cob flour and 50% sweet potato flour) added to the “Prozi” biscuit

formulation can increase the moisture content and ash content of biscuits.[21]

Organoleptic quality of pumpkin puree and katuk leaf substitution biscuits was assessed based on the results of organoleptic tests on 50 untrained panelists. The Kruskal-Wallis test results in Table 3 show that there are significant differences for all organoleptic test parameters conducted by untrained panelists. The Mann-Whitney test results showed that F1 and F2 were not significantly different for all parameters, but for F1 and F3 each significantly different in all organoleptic test parameters of pumpkin puree biscuits and katuk leaf substitution biscuits. The results of the organoleptic test for color parameters showed that there were significant differences in color in pumpkin puree and katuk leaf substitution biscuits (ρ = 0.002). This is due to the increasing number of katuk leaves substituted, the color of the biscuits becomes greener, due to the influence of chlorophyll pigments contained in katuk leaves. Green color is generally preferred by panelists, but in a product, aroma and taste parameters also determine besides texture. The higher the percentage of katuk leaves, the more dominant the green color of the biscuits.

The test results between biscuit formula treatments, it can be said that F1 (40% pumpkin puree: 3% mashed katuk leaves) is most preferred by panelists from the color parameter. In line with the research of Kusumawardani et.al. which states that biscuits with composite flour substitution (moringa leaves, seaweed and banana) of 25%, for all four parameters (color, taste, texture and overall) are most preferred by panelists compared to biscuits with composite flour substitution of 30%, 40%, and 50%.[22] Research by Putri, Almasyhuri, and Mirani, stated that although there were significant differences in cookies with the addition of milk and fat, there was no significantly different effect for F1 and F3 and vice versa F1 and F2 were significantly different.[23] The results of research by Muhammad Nu, et al, showed that the addition of katuk leaf flour and the addition of moringa leaf flour had a significant effect on the level of liking for the color of cookies (p=0.009).[24] The results of this study state that the substitution of other food ingredients in a product with a certain amount will affect the resulting product.

The results of the aroma parameter research show that there is a significant difference in aroma in pumpkin puree and katuk leaf substitution biscuits (ρ=0.005). This occurs because the greater the amount of substitute ingredients, especially katuk leaves, can cause an aroma that is less preferred by panelists. According to Matz and Matz 1978, aroma arises because during the roasting process the volatile compounds contained in the material evaporate. The aroma of biscuits is also caused by various components of other ingredients in the dough such as margarine, sugar. The developer material in making cookies functions as an aroma regulator. The results of testing the treatment biscuit formula, it can be said that F1 (40% pumpkin puree: 3% katuk leaves) is most favored by panelists from the aroma parameter. In line with Erdiyawati and Astuti's research which states that the aroma of Rich Biscuits with the addition of katuk leaf flour is savory and slightly languorous.[25] The results of research by R. H. Putri et al. showed that the aroma of biscuits based on local food ingredients (katuk leaf flour, pumpkin flour and corn flour) with the addition of soy flour in the t1 treatment was favored by panelists.[26]

The results also showed that there was a significant effect of pumpkin and katuk leaf substitution (ρ=0.011) on biscuit texture. However, when viewed from the average score of the panelists, the results varied for the three formulas. The texture of the biscuits in this study was influenced by the substitution of pumpkin puree and mashed katuk leaves which basically still contain water. However, by baking the biscuits using an oven at $130^0$C for 30 minutes, the water content in the biscuits can be said to meet the requirements in SNI 2973: 2011 for F0, F1, and F2 which is a maximum of 5%, but not for F3 biscuits. The texture of biscuits is closely related to the moisture content of a food product. If the moisture content is low, the texture will be crispier. The hardness of the biscuits is influenced by the biscuit formulation in this case the presence of substituted food ingredients or other food additives, the use of wheat flour and the thickness of the biscuits. In line with the research of Wihenti, et.al, the lower the moisture content of the biscuits, the crispier the texture produced.[27] The low moisture content of biscuits is caused by the oven temperature during the baking process. Research by Rianta, et al. also stated that the thickness of the biscuit also plays a role in the hardness of the cookies, the thicker the biscuit, the greater the force or strength to destroy the texture of the biscuit when consumed.[28] Thin biscuit

thickness affects the texture, resulting in a crispy biscuit texture.

The texture of pumpkin and katuk leaf substitution biscuits is also influenced by the decrease in wheat flour along with the increase in pumpkin and katuk leaf substitution in the biscuit dough. Wheat flour contains 80% protein known as gluten and gliadin. When these two types of protein mix with water or liquid milk in biscuit dough, it will form an elastic substance called gluten. Research by Ratna Yashinta, et al. showed that the texture produced from cookies was very crispy and favored by panelists. The crunchy texture of these cookies is influenced by the basic ingredients used, namely mocaf, which has a low protein content. Low protein will affect the glutenin and gliadin content of the protein, so that the resulting texture will be crispy and favored by panelists.[29]

Organoleptic quality with the flavor parameter of biscuits substituted with pumpkin puree and katuk leaves showed that there were significant differences in taste in untrained panelists (ρ = 0.000). The organoleptic quality of biscuits from the flavor parameter is determined by the nature and amount of ingredients used. Eight trials conducted by researchers proved that the characteristics and amount of ingredients used, especially substitute ingredients in biscuit dough, will affect the taste and aroma of biscuit products. Pumpkin puree and katuk leaf substitution biscuits generally provide a savory, delicious and crunchy taste, but soft when the biscuits are consumed. In line with the results of research by Muhammad Nu et al, which stated that the addition of katuk leaf flour and the addition of moringa leaf flour had a significant effect (ρ = 0.002) on the level of liking for the taste of cookies.[24] Research by R.H. Putri et al. also stated that there was a significant difference in the taste of local food-based biscuits (katuk leaf flour, pumpkin flour, corn flour) with the addition of soy flour.[26]

Organoleptic quality for pumpkin and katuk leaf puree substitution biscuits in general gives the results of F1 biscuits which have the highest acceptance from panelists with an average score of 4. The results of the overall acceptance of biscuit product parameters in this study are based on the summation of the overall panelist assessment score (all parameters), then F1 biscuits also get the highest score of 783. These results are in accordance with statistical tests which prove that there are significant differences between the parameters of color, aroma, texture and taste, each against the substitution of pumpkin puree and katuk leaves in biscuits. The limitation in this study is the type of panelists used, namely 50 untrained panelists, because untrained panelists do not represent broader consumer preferences and untraining can reduce standardisation in the sensory test process.

## CONCLUSION

The biscuits from this study can be an alternative snack choice for pregnant and lactating women, because they contain antioxidant compounds, namely phenols and flavonoids, so these biscuits also have antioxidant activity in protecting body cells from damage by free radicals, especially for pregnant and lactating women. The moisture content and ash content of the biscuits have met the standards of SNI 01-2973-1992, although further research is still needed for the shelf life and quality of the biscuits after storage. The F1 biscuit formula, containing 40% pumpkin puree and 3% katuk leaves, was the most preferred by panelists, receiving an average sensory score of 4 out of 5. The formula also achieved a total score of 783 across all sensory parameters, making it the most favorable choice in terms of taste, texture, color, and aroma. This research suggest to get the best substitution concentration from this study, it is proposed to use consumer panelists to test the acceptability of the product and conduct a shelf life test of this biscuit.

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