e-ISSN : 2621-6973
Jurnal **Pengolahan Pangan** 7 (2) 92-98, Desember 2022 p-ISSN : 2527-5631

NUTRITION AND GLUTEN ANALYSIS OF MODIFIED CASSAVA FLOUR-BASED COOKIES WITH WHEY PROTEIN CONCENTRATE ADDITION

ANALISIS KANDUNGAN GIZI DAN GLUTEN COOKIES TEPUNG MODIFIKASI SINGKONG DENGAN PENAMBAHAN WHEY PROTEIN KONSENTRAT

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ABSTRACT

Indonesia is one of the largest cassava producers in the world. Cassava can be processed into modified cassava flour (MOCAF) which has many functions. A lot of studies about MOCAF have been conducted to know the potency replacing wheat flour. One of the popular snack products made from wheat flour is cookies with high protein. This study aimed to see the potential of the MOCAF as a raw material replacing wheat flour to produce low-gluten soft cookies with the addition of concentrated whey protein to increase the protein value. This research used 2 types of formulations, MOCAF: wheat flour (30%:70% and 40%:60%) and the addition of whey protein concentrate with evaluation on protein content, fat content, water content, and gluten. This study showed that both types of formulations have the potential as a substitute for wheat flour to make soft cookies. All formulations can be claimed to be gluten-free products and the second formulation can almost be claimed to be a source of protein. The resulting cookies have the potential to reduce dependence on wheat flour and can be consumed by consumers who have gluten allergies or have high protein requirements.

Keywords: cookies, gluten, MOCAF, snack, whey protein.

ABSTRAK

Indonesia merupakan salah satu penghasil singkong terbesar di dunia. Singkong dapat diolah menjadi tepung singkong modifikasi (MOCAF) yang memiliki banyak manfaat. MOCAF telah dipelajari untuk diharapkan dapat menjadi pengganti tepung terigu. Produk olahan snack berbahan dasar tepung terigu yang digemari adalah cookies dengan protein tinggi. Penelitian ini bertujuan untuk mengetahui potensi MOCAF sebagai bahan baku pengganti tepung terigu untuk membuat cookies lembut rendah gluten dengan penambahan whey protein konsentrat untuk meningkatkan nilai protein. Pembuatan cookies lembut menggunakan 2 jenis formulasi MOCAF: tepung terigu (30%:70% dan 40%:60%) dan penambahan whey protein konsentrat dengan evaluasi pada kadar protein, kadar lemak, kadar air, dan gluten. Penelitian ini menunjukkan kedua jenis formulasi memiliki potensi sebagai pengganti tepung terigu untuk membuat cookies lembut. Semua formulasi dapat diklaim sebagai produk bebas gluten dan formulasi kedua hampir dapat diklaim sebagai sumber protein. Cookies yang dihasilkan memiliki potensi untuk mengurani ketergantungan tepung terigu dan dapat dikonsumsi oleh konsumen yang memiliki alergi gluten atau memiliki kebutuhan protein yang tinggi.

Kata kunci: cookies, gluten, MOCAF, snack, tepung singkong modifikasi.

Introduction

Indonesia was ranked the fourth-largest cassava producer in the world with 21 million tons produced in 2019, after Nigeria as the top producer, Thailand, and Brazil as second and third largest cassava producer in the world (Saleh & Widodo, 2007). In Indonesia, Lampung is the highest producer of cassava with more than 250,000-hectare plantation areas and more than 6.6 million tons produced in 2018 (Kementerian Pertanian, 2019). The utilization of cassava flour

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e-ISSN : 2621-6973
p-ISSN : 2527-5631

is still low, however, it has potency as a substitute for wheat flour when treated properly (Novitasari, 2017). Cassava can be processed into modified cassava flour (MOCAF) which has no gluten. Modified Cassava Flour (MOCAF) is flour originally processed from cassava tubers, which is fermented by lactic acid bacteria (LAB). MOCAF contains high carbohydrates and no gluten. Unlike wheat flour, MOCAF contains a very low protein level.

Recently, the demand for gluten-free food products has been increasing rapidly due to the increased global awareness of several health conditions: People who are allergic to wheat, coeliac disease, and non-coeliac gluten sensitivity. Wheat allergy commonly appears in children caused by an abnormal sensitivity to wheat protein (Husby et al., 2012), Non-coeliac gluten-sensitivity is an abnormal reaction that occurs during the ingestion of the wheat product (Catassi et al., 2013; Dahl, 1979), while the coeliac disease is a severe condition in the digestive system when certain people consume gluten-containing products (Husby et al., 2012).

The cookies which use modified cassava flour will have the low amount of protein while protein is one of the most important macronutrients for the human diet. High protein products are essential due to the increasing demand for healthy food products. Children in the growth phase need a higher amount of protein. Active adults such as bodybuilders also need a high amount of protein to build up and maintain muscle mass. Elderly people also need a high amount of protein, especially elder people diagnosed with sarcopenia, whose muscles shrink gradually (Beasley et al., 2013). Seeing those problems, high protein gluten-free cookies are a promising way to be studied since there are groups of people that have special needs.

According to Euromonitor International (2019), the trend of the protein bars in Indonesia is predicted to increase until 2023, and cookies consumption in Indonesia ranks second highest after chips (Jack, 2017). Cookies products are categorized as the same category with a snack bar.. Soft cookies seem a popular snack that tends to be accepted by many populations from children to elder people.

According to (Perdani et al., 2018) the highest overall acceptance of cookies is 100% wheat flour. Substitutions above 10% lead to a significant difference compared to the control (100% wheat flour). Another study also stated that the optimum amount of wheat flour:modified cassava flour is 75%:25% (Suhartatik and Widanti, 2018), whereas a study by (Normasari, 2010) stated that optimum amount of wheat:modified cassava flour percentage is 55%:45%, while study by Raharja (2018) and Pramadi et al. (2020) stated modified cassava flour can replace wheat flour 100%.

High protein soft cookies with modified cassava flour are a potential way to solve those problems. In addition, the usage of modified cassava flour may help to empower Indonesia's natural resources, soft cookies production using modified cassava flour can help achieve national food security.

Method

Material

Commercial materials were used in the experiment: wheat flour (Bogasari Kunci Biru), baking soda (Koepoe-koepoe), baking powder (Koepoe-koepoe), salt (Dolphin), granulated sugar, palm sugar, vanilla flavouring (Koepoe-koepoe), eggs, and margarine (Blue Band) were bought from a local market. Modified cassava flour (PT. Rasa Sayang Ais Krim) and whey protein concentrate (WPC) (Glanbia, USA) were bought through an online shop. The equipment were mixer, oven, analytical scale, cup, thermometer and laboratory glass tools.

Method

Cookies production

A general formula and making of cookies with some modifications were used in the experiment. The amount of WPC added was at a level that the cookies produced will contain at least 20% protein, to comply with the Peraturan Kepala BPOM No 13 Tahun 2016. Based on the producer claim that WPC contains 79.24% protein, the amount of WPC added was 191 g. The ingredients for cookies production were 340 g wheat flour, 191 g WPC, 4 g baking powder, 4 g baking soda, 5 g salt, 170 g margarine, 85 g granulated sugar, 85 g palm sugar, 1 egg, 100 g chocolate chip and milk chocolate chunk respectively. Two kinds of flour i.e MOCAF and wheat flour were mixed at different ratios, namely 30%:70% (formula A) and 40%: 60% (formula B) respectively.

In the first stage of mixing, the ingredients such as margarine, palm sugar and granulated sugar were mixed for approximately 15 minutes.

In the second stage of mixing, egg and vanilla flavoring were added and mixed for 1 minute. For the third mixing, MOCAF, wheat flour, baking soda, baking powder and salt were added. After the refrigeration process for around 1 hour, the dough was molded with approximately 20 g of each. The baking process took 15 minutes at 180^{O}C of temperature heat.

1. Moisture Content Determination

A gravimetric method was performed for moisture content determination (SNI 2973: 2011). The amount of water in the sample was calculated by the difference in weight between the initial sample and the final sample, multiplied by 100%.

$$\text{Moisture content (\%)} = \frac{((A+B)-C)}{B} X100\% \quad (1)$$

A = Empty container mass (g)
B = Sample mass (g)
C = Weight of container + sample after heating (g)

2. Gluten Content

An immunochromatographic assay Reveal® 3-D from Neogen was applied to determine the gluten level. The method is a combination of immunoassay and chromatography as shown in Figure 1 and 2 (Tankeshwar, 2020).

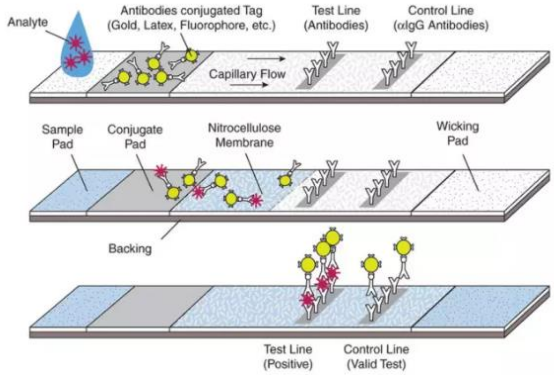

Figure 1. Immunochromatographic Assay (Paulini et al., 2017)

The sample was ground and mixed with buffer type 4. The sample is then mixed and agitated for 1 minute, before buffer extraction type 5 was mixed. The sample agitated again for 1 minute before the testing kit was dipped inside the tube. This kit can detect the presence of gluten in the food sample.

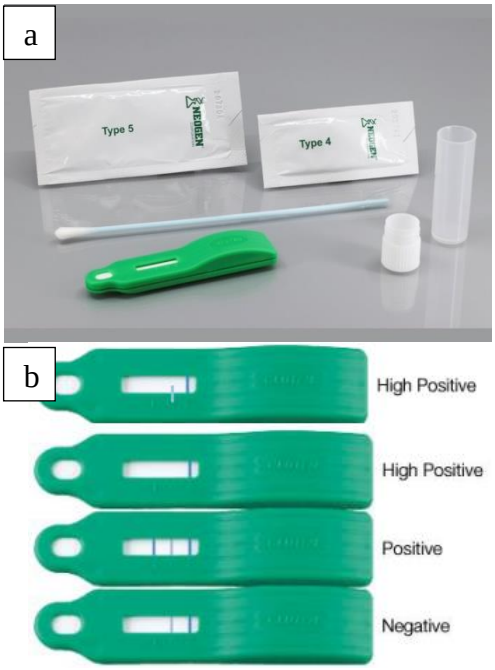

Figure 2 (a) Gluten Test Kit by Neogen and **(b)** Kit Reveal 3D Result Interpretation (*Reveal® 3-D for Gluten*)

By using Reveal® 3-D shown in Figure 2 (a) for Gluten from Neogen, the amount of gluten that exist in the sample can be known by observing the lines that appear on the tool as shown in Figure 2 (b). The kit will show a negative result if the amount of gluten present is below 5 ppm. Furthermore, the kit will give a positive result if the amount of gluten present is between 5-10 ppm. When the amount of gluten exceeds 10 ppm, it will give a high-positive result.

3. Crude Protein Content

The Kjeldahl method was used to determine the crude protein content of sample (AOAC 2001.11. 2005) with a factor of 5.70. The Kjeldahl method consists of three main steps: digestion, distillation, and titration. The amount of nitrogen then calculated by using the following formula:

$$\text{Protein content (\%)} = \frac{((Vp-Vb)XNX1.4007XF}{\text{sample mass (g)}} X100\% \quad (2)$$

Vp = The volume of HCl needed for sample titration
Vb = The volume of HCl needed for sample titration
N = Normality of HCl 0.2N

Where the F is the factor to convert N into protein. According to SNI 2973:2018, the F factor for cookies is 5.70.

4. Crude Fat

The level of crude fat was determined Soxhlet method using hexane as a solvent (SNI 2354-3-2017) Lipid content determination was using the gravimetric method. This method requires organic solvent (hexane) to dissolve and extract fat from the sample to be analyzed. The amount of lipid was calculated by the following formula:

$$\text{Crude fat content} = \frac{\text{Mass of fat (g)}}{\text{Mass of sample (g)}} \times 100\% \quad (3)$$

Statistical design

A completely factorial randomized design was used in the experiment which is run in duplicate. This type of research is quantitative research, specifically experimental research design. The experimental method was chosen because this research method is able to find the effect of certain treatments with under controlled conditions. The study was performed using the factorial completely randomized design with 2 replications (duplo). Statistical analysis was performed by using the one-way analysis of variance (Anova) was determined at $p<0.05$, followed by a least significance difference. and the mean were compared across group and the significant differences were determined at $p<0.05$.

Results And Discussion

Results analysis of cookies is shown in Table 1, with 4 parameters, which are moisture content (%), total fat (%), protein content (%), and gluten level. The references 100% wheat flour and commercial cookies are also presented for the comparison.

It has been explained that modified cassava flour can substitute wheat flour in various of percentages, certainly with different cookies characteristics. To increase the percentage of modified cassava flour that can be substituted, other additional ingredients are needed to fix the problems that arise if the modified cassava flour is used in large quantities.

According to SNI 2973:2018, cookies is classified as a type of biscuit made from soft or crunchy dough and when broken, the appearance is less dense compared to biscuits. The standard of cookies is defined in table 1. According to Peraturan Kepala BPOM No 34 Tahun 2019, the definition of cookies defined as the same as elaborated in SNI 2973:2018. However, an additional requirement for soft cookies is regarding the water content. Soft cookies water content should remain between 5-10%.

Laboratory analysis has been conducted to analyze moisture content for 2 sets of samples (formulation A and formulation B) which contained 30% and 40% of modified cassava flour respectively. Formulation A contained 11.21% moisture while formulation B contained 11.80% moisture. Table 1 showed the statistical differences of moisture content data for both formulations. Both formulations exceeded the maximum moisture allowed by BPOM (10%) and SNI 2973:2018. The high level of moisture content in these cookies may be explained as the temperature of baking is not stable enough. This research used 180^{o}C, while another study stated that the temperature can be raised until 220^{o}C to produce good quality cookies with a low level of moisture content (Davidson, 2019a).

Table 1. Result analysis of cookies

Parameter	Unit	Result			
		Formulation A(1)	Formulation B(2)	100% wheat Flour Cookies(3)	Cookies Standard(4)
Moisture content	%	11.20 + 0.01[a]	11.81 + 0.12[b]	12	Max 10
Total fat	%	23.00 + 0.90[a]	21.52 + 0.09[a]	1.5 - 2	-
Protein content	%	17.75 + 0.23[a]	19.65 + 0.33[b]	8 - 13	Min 4.1
Gluten	ppm	5-10	5-10 ppm	No data	-

Means within a column followed by different letters are significantly different at $P<0.05$.

(1) MOCAF: wheat = 30%: 70%
(2) MOCAF: wheat = 40%: 60%
(3) Subagio, 2006
(4) SNI 2973:2018

e-ISSN : 2621-6973
p-ISSN : 2527-5631

Another reason is the type of packaging was not airtight. As the result, moisture from outside could enter the packaging while waiting for the analysis in the laboratory. The other potential reason is caused by inadequate baking time.

Fat is ultimately needed to make good quality cookies as fat has functions to improve the taste and create a softer texture of cookies. Sufficiency of fat is needed to give excellent quality of soft cookies. The fat in these cookies is coming from margarine, egg, chocolate chip and milk chocolate chunk. In SNI 2973:2018, there is no standard for the total fat amount. Fats also tend to inhibit the leavening action of the carbon dioxide diffusion in the dough during baking, and this produces a softer, finer texture. Where both fat and sugar amounts in the recipe are high, they combine to make a soft, syrupy, chewy texture (Davidson, 2019b). The finding of this study found the average fat content in soft cookies is 22.26%, thus the possibility of oxidation is high. It is suggested to use the addition of antioxidant such as BHA/BHT to delay the oxidation process.

Lipase and lipoxygenase are commonly present in wheat. During processing, the cell will be damaged and causes contact between lipids and enzymes, causing the appearance of rancid odors. Not only the presence of those enzymes, type of fat that is added to the cookies also influence the shelf life. This high-level of fat may affect the shelf life of this product. However, there is no statistical difference between formulation A and B

Table 2. Gluten-free and Low-Gluten Claim Adapted from Peraturan Kepala BPOM nomor 13 tahun 2016

Parameter	Unit	Requirement (ppm)	
		Gluten-Free	Low-Gluten
Gluten	mg/kg	≤ 20	21 - 100

According to Peraturan Kepala BPOM no 13 Tahun 2016, it has been regulated the requirement to achieve low gluten claim, while the maximum gluten amount for a low-gluten claim is 100 mg/kg or ppm and for a gluten-free claim is 20 mg/kg or ppm as shown in Table 2. Laboratory analysis regarding gluten was analyzed by using Reveal® 3-D gluten test. A positive result will appear if there are 5-10 ppm gluten in the sample. Since both formulations are in the range of 5-10 ppm for both formulations, not only low-gluten claim can be achieved but also the gluten-free has been achieved. However, this research cannot quantify the amount of exact gluten presented in the cookies. The better method to quantify the gluten level as regulatory compliance of gluten-free products is enzyme-linked immunosorbent assays (ELISAs), which are using the antibodies, either monoclonal (mAb) or polyclonal (pAb), for example, the R5 (Lexhaller et al., 2017).

According to BPOM claim regulation as shown in table 3, the minimum amount of protein was 20% per 100g in solid form to get the "source of protein" claim. The protein contribution was not only coming from protein concentrate, but also from the wheat flour and egg white that were used in the recipe.

Table 3. High and Source of Protein Claim Adapted from Peraturan Kepala BPOM nomor 13 tahun 2016

Component	Claim	Requirements not less than
Protein	Source	20% per 100 g (in the form of solid) or 10% per 100 ml (in the form of liquid)
	High/Rich	35% per 100 g (in the form of solid) or 17.5% ALG 100 ml (in the form of liquid)

Based on the lab result, the average protein content from formulation A and B is 17.76% and 19.65% respectively as presented in table 1, therefore statistically differences were found protein content in all formulations did not achieve the minimum requirement from BPOM to claim source of protein (20%) therefore, this product cannot be claimed as protein source product. The probable reason was from the miscalculation results of recipe formulations, particularly the whey concentrates calculations with analysis of whey protein concentrate content might be lower than 79.24%. Another reason is the usage of improper balance. Thus, it affects protein content at the final result. Theoretically, formulation B should have lower protein content than formulation A, however, the opposite result happened.

Protein, together with carbohydrates and lipids are the basic macronutrient in human diet.

Recommended Dietary Allowance (RDA) value for both men and women is 0.80 gram/kg body weight/day (Lupton et al., 2002). Addition of protein in cookie product is preferable as protein carrier since cookies have a long shelf life and high acceptability. However, in general, protein content in cookies is low, thus the influence on the final product has not been discovered. A study by Sahagún and Gómez (2018) aims to analyze the influence of protein type and percentage of flour substitution towards dough rheology, cookie characteristics, overall cookie quality and overall acceptance among consumers.

Conclusion

The effort to optimize the utilization of cassava should be conducted as one of the mitigations to ensure the food security by replacing the wheat flour which is being imported from outside of Indonesia. Protein and moisture content were not significantly different. The gluten-free claim has been successfully achieved for both formulations. Modified cassava flour has a potency to replace 100% of wheat flour. Future research is obviously needed to find lower moisture content as the requirement from the SNI for cookies. In addition, sensory analysis is also required to find out the acceptance and preference level of consumers.

Acknowledgments

The authors would like to thank many colleagues for their valuable help with these experiments. This research is funded by Faculty Research Fund, Swiss German University.

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