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The Potential of Lipid Oxidation on Non-Gluten Mocaf Cookies Incorporated with Chicken Meat-Carrot Puree

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ABSTRACT: Cookies and crackers are the most popular snack in many countries. They are essentially made from wheat flour, which is also an issue as Indonesia is completely dependent on wheat imports. On the other hand, people with celiac disease could not consume wheat-derived products, including biscuits. Replacing the raw material of biscuits with local flour is one approach to reduce the use of wheat and help people with celiac disease. The aim of this study was to evaluate the quality of cookies made from modified cassava flour (mocaf), incorporated with chicken meat and carrot puree, from the point of view of lipid oxidation potential. The parameters observed were free fatty acids, peroxide value and thiobarbituric acid (TBA) number as indicators of lipid oxidation. The experimental design used was a completely randomised design with one factor, the ratio of chicken meat to carrot puree. The ratio was in 4 levels (F1 = 0:0 (control), F2 = 12.5%:37.5%, F3 = 25%:25%, F4 = 37.5%:12.5%). The results showed that the free fatty acid content had a range of 4.37-5.47 g/100 g, peroxide values 2.73-5.75 mq.eq/kg and TBA values 4.96-5.46 mg.mlonaldehyde/kg. Compared to the sample control (F1), the F4 formulation was considered to be the best biscuit in terms of low peroxide value and TBA values.

KEYWORDS: Cookies, Mocaf, Free Fatty Acid, Peroxide Value, TBA.

1. INTRODUCTION

Coeliac disease is a chronic inflammatory disorder of the small intestine caused by the ingestion of dietary gluten products in susceptible individuals. Gluten is a specific protein found in wheat and wheat-based products. The common food in market containing gluten are bread and pastries, cookies, and noodles. In developing countries, the number of cases of coeliac disease is increasing as people tend to have modern lifestyle. It affects their habit for choosing gluten food on daily basis (1). Conversely, it is now recognized that undiagnosed coeliac disease can have serious consequences in both children and adults.

Cookies and crackers are the most consumed snacks in many countries. The word "cookies" is used in United States, while in United Kingdom it is called "biscuit". It is essentially made from wheat flour. It is an ongoing issue since Indonesia is fully dependent on wheat imports. Foreign Agricultural Services (2022), showed information on wheat imports for 2021 to 2022, is raised to 11.2 million metric tons (MMT) from the previous estimate of 11.0 MMT(2). This reflects an increasing demand and consumption of flour-based foods. However, people with coeliac disorder are unable to consume processed food products from wheat. Substituting raw material of cookies from local flour is an approach to reduce the usage of wheat and to help coeliac sufferers (3).

Modified cassava flour (mocaf), is a flour made from cassava by using microbial fermentation techniques. It has similar physical and chemical properties to wheat flour. Due to the properties of mocaf, which is white in colour, mocaf can be used to substitute different types of flour. Historically, mocaf was first produced to replace wheat flour. Unfortunately, mocaf is low in protein content (1.2%), fat (0.4%), and mineral as represented by ash (0.4%) (4). The addition of protein materials in the cookies is important to improve the nutritional value. Chicken meat is considered as a high-quality protein source (30.9%) and contains factors that promote the bioavailability of a variety of nutrients, which is hence often larger compared to that of the same nutrients present in plant-based foods(5).

Enhancing the beneficial health of cookies is possibly done by combining vegetable sources as ingredients to optimize phytochemical fibre content within (6). Carrot (*Daucus carota* sativa), is a rich source of β-carotene, a precursor of vitamin A and a proven antioxidant (7). Antioxidants in vegetables play a vital role in the prevention of oxidative stress-related diseases. With the heat-stable carotenoid pigment properties, carrot is suitable to be incorporated into a baked product.

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Cookies hold a key position in the snack market due to their variety of flavours, crispiness texture, ready-to-eat properties, availability, and longer shelf life. Among the ingredients of cookies, fat is the component that responsible for the rich, creamy taste, and it interacts with other components in forming specific texture of the product. On the other hand, fat oxidation is the main cause of food quality deterioration and has a big impact on food attributes (flavour, colour, texture, nutritional value, and food safety. The present study was conducted to determine the potential of lipid oxidation by indicators of free fatty acid, peroxide value, and thiobarbituric acid on non-gluten mocaf cookies incorporated with chicken meat and carrot puree.

2. MATERIALS AND METHODS

2.1 Biomaterials

Modified cassava (mocaf) flour was brought from Yogyakarta, Indonesia. The chicken meat, carrots, and all other ingredients which include sugar, salt, butter, baking powder were obtained from Blitar City, East Java. The chemicals used included H2SO4 (Emsure), Kjeldahl powder, HCl (Sigma), NaOH (Merck), Alcohol 96% (Mercks), K2SO4 (Sigma), N-Hexane (Emsure), and distilled water.

Modified cassava flour (mocaf) was sourced from Yogyakarta, Indonesia. Chicken meat, carrots, and all other ingredients including sugar, salt, butter and baking powder were obtained from Blitar City, East Java. Chemicals used were ethanol (Merck), phenolphthalein indicator, NaOH (Sigma-Aldrich), acetic acid (Merck), Chloroform (Merck), KI solution, Na2S2O3 solution, amylum powder (Merck), HCl (Merck), TBA reagent, and aquadest.

Instruments and tools used were spectrophotometer (GENESYS 10 UV-Vis), analytical balance (IBA-8600), pH meter (Benchtop PH 700), waterbath shaker (WSB-30), distillation glassware, Erlenmeyer flask 250 ml (Pyrex), hotplate (IKA HS-7), stopwatch, filter paper (Whatman 41), volumetric flask (Pyrex), pipette, and burette.

2.2 Sample preparation

2 kg of chicken fillets were cleaned and steamed to achieve a compact texture. Cooked chicken meat was ground using a food processor (Philip HR-7310) and roasted into a dried texture. Carrots were peeled, washed, and chopped into 2 cm long prior to water blanching (120 secs). Blanched carrots then immediately put into cold ice water. The carrots were smoothed to a thick puree with juice blender (Philips HR-2115).

Cookies production was started by mixing the 2 beaten eggs and 50g sugar until the mixture thickened homogenously pale yellow. 8g of salt, 2 g of baking powder, and 120 g butter were added and the dough continuously mixed. The carrot puree, chicken meat, and 200 g of mocaf flour was added after. The ratio of chicken meat:carrot puree was at 4 levels, those were F1 = 0:0 (control), F2 = 12.5%:37.5%, F3 = 25%:25%, F4 = 37.5%:12.5%.

The dough rolled into walnut-sized balls and placed on a baking sheet that has been lined with baking paper. Cookies dough balls then flattened and baked in an oven (Modena BO-2664) at 170ºC for 40 minutes. Cookies were cooled and stored in aluminum foil pouch bags before being tested for its potential of lipid oxidation by parameter of free fatty acid, peroxide value, and thiobarbituric-acid number by method based on Emeline et al., 2020(8).

2.3 Determination of free fatty acid

5g of crushed cookies samples weighed and placed into 250 mL Erlenmeyer flask. 50 mL of hot ethanol and 2-3 drops of phenolphthalein indicator were added. Sample solution titrated with NaOH 0.1 N until the pink color obtained and not disappeared along 30 secs. The percentage of free fatty acid (FFA) calculated as:

$$\%FFA = \frac{ml\ NaOH\ x\ N\ x\ MW\ fa}{sample\ weight\ (g)\ x\ 1000}\ x\ 100$$

N : normality of NaOH solution
MW fa : molecular weight of fatty acid

2.4 Determination of peroxide value

5 g of grinded sample was placed in a 250 ml erlenmeyer flask and 30 mL of acetic acid-chloroform (3:2) was added. The solution was homogenized and 0.5 mL of saturated KI solution was added. Sample then placed on a waterbath shaker for 1 min and 30 mL of distilled water was added. The sample was titrated with 0.1 N Na2S2O3 until the yellow color tend to disappeared. 0.5

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mL of 1% amylum solution was added, then titrated again until the blue color mostly disappeared. The peroxide number expressed in milli-equivalents of peroxide in each kg of sample.

$$P = \frac{ml\ \text{Na2S2O3}\ x\ N\ x\ 1000}{sample\ weight\ (g)}$$

P : peroxide value
N : normality odf Na2S2O3

2.5 Determination of thobarbituric acid (TBA) number

3 g of cookies crushed sample added with 50 mL of aquadest. The sample solution was transferred into a 1000 mL distillation flask with 48.5 ml distilled water added. 1.5 mL of 4 N HCl was added until the pH became 1.5. Boiling stone and froth prevention agent (antifoam) were added to the sample solution for further distillation. Distillation was carried out with the highest possible heating so that a distillate of 50 ml was obtained during 10 minutes of heating. The distillate was filtered and transferred 5 mL into a 50 mL erlenmeyer flask with a lid and 5 mL of TBA reagent (0.02 M thiobarbituric-acid solution in 90% glacial acetic acid).

The dissolution was accelerated by heating on a waterbath shaker for 35 mins. After cooling, the optical density value was measured with a spectrophotometer at a wavelength of 528 nm. A blank solution was used as a comparison. The TBA number was calculated and expressed in mg malonaldehyde/kg sample. The calculation of the TBA number was:

$$TBA = \frac{3\ x\ A\ 528\ x\ 7{,}8}{sample\ weight\ (g)}$$

3 : iodine number as the value of the degree of unsaturation of oil/fat
A 528 : absorbance at wavelength 528 nm
7.8 : TBA number conversion rate mg/malonaldehyde/kg sample

2.6 Statistical analysis

All samples were run in triplicates. The data were expressed as mean ± standard of deviation. Variation of data were analyzed using one-way analysis of variance (ANOVA), followed with Duncan's multiple-range test to separate means value. The significance was analyzed at the level of 0.05 ($p < 0.05$) between samples.

3. RESULTS AND DISCUSSION

3.1 Free fatty acid content

Cookies hold a key position in the snack market due to their variety of flavors, crispiness texture, ready-to-eat properties, availability in different shape or dimension, also longer shelf life (7). Among the ingredients of cookies, fat is the component that responsible for the rich, creamy taste, and it interacts with other components in forming specific texture of the product. On the other hand, fat oxidation is the main cause of food quality deterioration and has a big impact on food attributes (flavor, color, texture, nutritional value, and food safety). Within fat compounds, free fatty acids are strong prooxidants in both bulk and emulsified oils .

Table 1. Profile of free fatty acids, peroxide value, and thiobarbituric-acid numbers on non-gluten mocaf cookies

Parameters	F1	F2	F3	F4
FFA g/100g(%)	5.12 ± 0.10b	5.47 ± 0.05a	5.20 ± 0.12b	4.37 ± 0.12c
Peroxide value (mq.eq/kg)	2.73 ± 0.04d	5.75 ± 0.02a	4.68 ± 0.04b	3.11 ± 0.02c
TBA (mg.malonaldehyde/kg)	5.33 ± 0.02b	5.46 ± 0.01a	5.42 ± 0.02a	4.96 ± 0.03c

Expressed values were mean±SD (n = 3); a same rows containing means with the same superscript letters are not significantly different ($p < 0.05$).

In mocaf cookies incorporated with chicken meat and carrot puree, the content of free fatty acids was significantly different ($p<0.05$). F2 had the highest value (5.47%) and F4 had the lowest value of FFA (4.37%) (Table 1). The major fat sources in mocaf cookies were from butter. Butter has fat content 82% (USDA, 2010). Regarding the ratio of chicken meat:carrot puree F2 was given 12.5%:37.5%, meanwhile F4 was 37.5%:12.5%. During baking, exposure to high temperature, oxygen, and moisture might lead to

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the hydrolysis product from fat such as free fatty acid(9). These FFA scores (Table 1) were much lower than FFA values in various biscuits investigated by Kandhro et al., 2008 (10), which ranged from 13.69-27.64 g/100g. Also, FFA content of mocaf cookies in this paper were lesser than in Turkish biscuits that reported to have range from 8.5-26% (11). There is no universal standard for the acceptable level of FFAs in cooked food such as cookies. But, for originally animal fats, such as lard, tallow, or butter, the FFA content should not exceed 1% (as oleic acid) for fresh fat, and 2% (as oleic acid) for used fat. Higher level of FFA indicates a higher level of fat/oil hydrolysis (10).

Free fatty acids (FFAs) are released by the action of lipases from various sources in dairy products. Lipases in butter can originate from those endogenous to milk, from the growth of starter and non-starter bacteria, and mainly from heat-stable enzymes (12). Excessive release of volatile FFAs in butter can lead to hydrolytic rancidity resulting in off-flavor that described as rancid, butyric, goaty, soapy, unclean, astringent and bitter (13).

3.2 Peroxide value

Peroxide value is a measure of the oxidation or rancidity of fats and oils. It is indirectly mention the amount of peroxide oxygen or active oxygen per 1 kilogram of fat or oil. Peroxide value represents the primary product of fat oxidation. It can be used as a determinant of oxidation status (14). The peroxide value from all four cookies samples were significantly different (Table 1). F2 had the highest value (5.75 m.eq/kg) and F1 had the lowest value of peroxide (2.73 m.eq/kg). Regarding the composition, F2 had the most proportion of carrot puree addition (37.5%), whereas F4 had the minimum carrot puree (12.5%). These result was above than Nagarajaiah & Prakash, 2015 (7), that found rancidity by peroxide value on carrot pomace-incorporated cookies. It was increased from 0.09 to 0.24 meq/100 g in control samples and in 4, 8, and 12%, the peroxide values ranged from 0.09 to 0.23 meq/100 g samples during storage.

Lipid oxidation resulting in the formation of peroxide compounds. It is triggered by a free radical free radical that took away the electrons from lipids in cell membranes. The cell being damage and the free radicals chain reaction continued. The initiator of free radicals in living cells are reactive oxygen species (ROS), such as OH- and HOO-. They merge to a hydrogen atom to form water and a fatty acid radical. The next thing happen is the fatty acid radical reacts readily with molecular oxygen to form a peroxyl fatty acid radical. This radical is also an unstable species that reacts with another free fatty acid to form another fatty acid radical and a lipid peroxide, or a cyclic peroxide if it has reacted with itself. This cycle continues as the new fatty acid radical reacts in the same way.

The primary oxidation of fat products in form of hydroperoxides, are relatively stable at room temperature. However, it is easily converted into alcohols, aldehydes, ketones and other secondary oxidation products. These products make cooking oils less acceptable to consumers or unacceptable due to poor taste. In addition, lipid oxidation has a negative effect on the nutritional aspect that might produce toxins which potentially harmful to the human body. Consumption of fat and oil products containing hidroperoxides can cause food poisoning and certain diseases (15).

3.3 Thiobarbituric acid (TBA) number

In case of lipid oxidation, peroxide value only indicates the amount of primary products. Later then, it easily broken down into secondary products, that peroxides may not reflect the actual extent of lipid oxidation. This, TBA is used to quantify the secondary oxidation products. Specifically, TBA measures the concentration of malondialdehyde (MDA), a secondary reaction product and a reactive aldehyde (16). TBA number on mocaf cookies incorporated with chicken meat and carrot puree was on the range from 4.96-5.46 mg.malonaldehyde/kg. These result was higher than TBA number on the pastry product, investigated by Hunaefi & Ulfah, (2019) (17), with the level below 1%. In addition, on the product of sponge cake made from white and brown rice by Dewi (2021) (18), which mentioned that TBA number of sponge cakes of each type of ranged from 0.039-0.054 mg malonaldehyde/g, with the highest TBA number shown in white rice sponge cake and the lowest shown in white rice sponge cake. Higher number of TBA in this paper might caused by the quality of butter (fat) used.

Furthermore, F2 had the highest TBA number, with the composition of 12.5% chicken meat and 37.5% carrot puree. On contrary, F4 had the lowest TBA number with 37.5% chicken meat and 12.5%. The interaction between fat and vitamin minerals is not a direct cause of rancidity. However, the presence of certain minerals such as iron and copper can catalyze the oxidation of unsaturated fatty acids in lipids, leading to the formation of hydroperoxides and other volatile compounds that contribute to rancidity,

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including malonaldehyde. USDA Food Composition Databases showed that chicken meat contains 0.7 mg of iron and 0.21 mg copper per 100 grams of raw meat, whereas carrot contains 0.4 mg of iron and 0.05mg of copper.

Malondialdehyde (MDA) has been associated with off-flavor and off-tastes in meat products. Thiobarbituric-acid (TBA) is a widely used reagent to measure the concentration of malondialdehyde (MDA), which is a secondary reaction product and a reactive aldehyde (19). Thiobarbituric-acid reactive substances (TBARS) are generated as a by-product of oxidative damage to lipids (i.e. fat degradation) and can be detected through the TBA assay with specific reagent. This method provides an indirect measure of ROS. TBA reacts with malondialdehyde (MDA), which is one of several low-molecular weight end products produced by the decomposition of some primary and secondary lipid peroxidation products (20).

4. CONCLUSION

The present study denoted that mocaf cookies incorporated with chicken meat and carrot puree had free fatty acids content from range of 4.37-5.47 g/100 g, peroxide values 2.73-5.75 mq.eq/kg, and TBA numbers 4.96-5.46 mg.mlonaldehyde/kg. Compared to sample control (F1), F4 formulation considered as the best cookies regarding the low peroxide value and TBA numbers. A further study about lipid oxidation of mocaf cookies products during storage is necessary.

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