Antioxidant properties, functional components, and sensory profile of green tea oatmeal cookies

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Abstract. As a healthy snack, oat cookies (OC) are very popular. During its development, OC was fortified with other ingredients to improve functional quality, such as green tea, which is high in antioxidants, particularly flavonoids. Previous research has shown that OC-fortified green tea has chemical properties specifically in accordance with a healthy diet (high in fiber but low in fat). Thus, this study aimed to evaluate catechin, epigallocatechin gallate (EGCG), antioxidant activity, and sensory properties of green tea oatmeal cookies (GTOC) compared to commercial and control cookies. The five cookie samples tested included oat cookies (control), wheat cookies, GTOC 6%, GTOC Commercial A, and GTOC Commercial B Antioxidant activity was measured using the DPPH assay, while catechin and EGCG contents were analyzed using LC-MS with 4-hydroxybenzoic acid as an internal standard. β-Glucan content was determined through extraction, purification, and characterization using LC-MS. Nutritional composition, including moisture, ash, fat, protein, and carbohydrate content, was analyzed using proximate analysis, and sensory evaluation was conducted with 25 panelists using a 4-point hedonic scale. GTOC 6% had significantly higher catechin, EGCG, and antioxidant activity than other samples. Sensory evaluation showed that GTOC was the most preferred by panelists in terms of taste, texture, and overall acceptability. The results suggest that green tea fortification enhances the functional and sensory properties of oat cookies. Green tea fortification in oat cookies improves antioxidant content while maintaining high consumer acceptability. Therefore, green tea fortification in oat cookies could enhance antioxidant activity and functional properties while maintaining high consumer acceptability.

Key words: antioxidants, functional foods, green tea, oatmeal cookies, health benefits

Introduction

Oatmeal Cookies (OC) is now a popular healthy snack among the public. This is due to the high fiber content of OC. Oat fiber is classified into two types: soluble fiber and insoluble fiber. The soluble fiber in oats is known as beta-glucan, and it has been shown to lower total cholesterol and Low-Density Lipoprotein (LDL) levels in the body (1). Unfortunately, OC is less appealing to some people because of its pale color. That is why some natural ingredients are enhancers for physical product quality, often fortified in the production process to improve its appearance without reducing its quality as a functional cookie. In the previous study, green tea (GT) powder was chosen as a food additive in OC making process to ameliorate its quality.

Green tea contains many more antioxidants than other tea types, known as polyphenols (2). Catechins, especially epigallocatechin gallate (EGCG), are a group of flavonoid polyphenolic compounds with the most significant number in GT. These are known to reduce low-density lipoprotein (LDL) levels, which is cholesterol, reduce the risk of cardiovascular disease, burn fat, and increase metabolism (3). The addition of GT powder indicated that the level can increase the antioxidant activity of cookies (4). Moreover, GT powder fortification improved the oatmeal cookie's functional properties. The addition of 6% GT in OC dough obtained a new variant product, namely green tea oatmeal cookies (GTOC), which not only brought in the greenness of the products but also better in nutrition facts such as low in fat and carbohydrates but high in fiber and protein content (5). On the other hand, further data related to the chemical characteristics, the functional component of GTOC, and other commercial cookies have not been available till nowadays. Some brands of GTOC products have been found in the market in recent years. However, information validity regarding its functional components must be disclosed further.

This study aimed to get more insight into the facts of nutrition, functional components (antioxidant activity, catechin, EGCG, and beta-glucan level), and sensory value (taste, aroma, texture, color, and hedonic) of GTOC resulted in this study compared to the other category of cookies such as wheat cookies, oatmeal cookies, and commercial GTOC-1 and GTOC-2. GTOC resulted in this study was expected to be a promising functional cookie in the future.

Materials and Method

GTOC preparation

The ingredients of GTOC were matcha powder (100% matcha), oatmeal, margarine, refined sugar, eggs, vanilla flavoring, all-purpose wheat flour, baking soda, and salt. First, the ingredients were mixed for 5 mins, and then the matcha powder (6% w/w) was added. The dough was then baked at 180° for approximately 30 mins. Then, GTOC was chilled for 10 minutes at room temperature.

Determination of antioxidant activity

Testing of antioxidant activity refers to one method, namely 2,2-diphentyl-1-picrylhydrazil (DPPH). Sample testing was carried out by means of 50, 100, 150, and 200 μ L pipetted using a micropipette, then, each sample concentration was inserted into the test tube, and DPPH μ M 3.8 ml was added in the vortex. The sample is left in a dark place for 30 minutes. The absorbance of the sample was measured at a wavelength of 515 nm with a UV-Vis spectrophotometer (6). The value of each sample concentration's antioxidant activity (IC50) is determined according to the quadratic equation y = a + bx. The value of x is the concentration, and y is the sample's absorbance (7).

Measuring of catechin and EGCG content

First, raw green tea catechins were extracted from cookies, then the catechins were analyzed for their active contents (EC, EGCG, EGC, ECG) using LC-MS (8). Catechin derivatives were analyzed using 4-hydroxybenzoic acid as an internal standard (I.S.). Mobile phase A used 1% CH3COOH:CH3OH (ratio 95:5) and a ratio of 5:95 as mobile phase B, with a gradient between 10% B to 55% B for 30 minutes at a flow rate of 0.2 mL/min. Nitrogen was used as a carrier gas with an output pressure of 0.5 Mpa and an ion source inlet pressure of 0.39 Mpa. The calibration curve was obtained from a linear regression of the peak area ratio of catechin/I.S vs. concentration, then the regression equation obtained was used to calculate the concentration of catechin. The calibration curve ranged from 3.125 µg/mL to 50 µg/mL for EC, EGCG, EGC, and ECG. The samples were ionized by SSI (Sonic Spray Ionization).

Measuring of β -glucan content

Beta-glucan is a polysaccharide that also acts as dietary fiber for the human body. It is abundant in oatmeal and functions to control cholesterol. Beta-glucan testing is carried out in several stages: sample extraction, purification, and characterization (9). The test will be analyzed using an LC-MS tool (10). Extraction was carried out using distilled water. The sample was

dissolved in distilled water, then the pH was adjusted using 20% sodium carbonate to reach pH 10. Extraction was carried out for 30 minutes in a shaking incubator at a temperature of 45 °C and a vibration of 125 rpm, then centrifuged to obtain the supernatant. The extraction process was carried out twice for each sample then the pH was lowered with 2 M HCl to reach pH 4 to precipitate the protein. The supernatant obtained was added with 50% isopropyl alcohol and then dried using a drying oven at 60 °C for 24 hours to obtain the crude β -Glucan sample. Purification of β -Glucan was carried out by dissolving crude β -Glucan with a concentration of 0.3% in distilled water and then dispersing it by heating it until it boils, then adding 30% ammonium sulfate. The dispersion solution was heated and then dialyzed using a membrane tube. The retentate was adjusted to a pH of 6.9 and then reacted with the enzyme α -amylase at a temperature of 40 °C for 1 hour. After the enzyme was inactivated, the solution was centrifuged, and the supernatant was dialyzed to obtain purified β-Glucan. Purified β-Glucan was set in electrospray with a flow rate of 800 μ L/min and an injection volume of 10 µL. The eluent used was 50% acetonitrile in deionized water. The fragmentor voltage was set at 150 V, and the capillary voltage was 4 kV. The temperature was maintained at 350 °C. The detection range was from m/z 100 to 1500.

Determination of nutrition facts

Proximate analysis is a laboratory method used to determine the macrochemical composition of a food or organic material sample. This method helps in understanding the main components in a sample, such as water, ash, fat, protein, and carbohydrate content. Water content was analyzed using the thermogravimetry oven drying method (11). The sample was weighed and then dried using an oven at 105 °C until the weight was balanced. The water content was calculated based on the initial and final weight of the sample, assuming that the result was the water content in the sample that evaporated during heating to change the weight of the sample. Ash content was analyzed using the dry ashing method (12). A total of 5 g of sample was put into a porcelain cup, ashed in a furnace with an initial temperature of 300 °C, and raised to 420 °C for 5 hours. The ash obtained was calculated in percent based on the initial and final weight of the sample without the cup. Fat content was analyzed using the Soxhlet method (13). A sample of 2 g was weighed and wrapped in filter paper, then inserted into a soxhlet series. Extraction with ether solvent and heating at 135 °C was carried out for 2 hours. The filter paper was then oven-dried until balanced. The fat content was calculated based on the initial and final weight of the sample in the filter paper. Protein levels were analyzed using the Kjeldahl method (14) in three stages: destruction, distillation, and titration. 1 g of sample was destroyed in a Kjeldahl flask with 10 ml of H2SO4 until it turned green. After the solution was cold, 100 ml of distilled water and NaOH were added. Then, distillation was carried out with a boric acid trap solution. Titration was carried out with 0.1 M HCl until a color change occurred. Protein content was calculated by converting the amount of nitrogen captured. Carbohydrates are calculated using the by-difference method by subtracting 100% from the water, ash, fat, and protein content. Total energy is calculated based on (15) by adding the results of multiplying the protein content by 4, carbohydrates by 4, and fat by 9.

Sensory properties

Sensory testing is a method to evaluate the organoleptic properties of a product or material using the five human senses: sight, smell, taste, hearing, and taste. This sensory test was conducted using 25 panelists who assessed 5 test parameters with different parameter scales. This test was carried out by giving a taste scale value (1-4 = not sweet - sweet), color (1-4 =not greenish - greenish), aroma (not floral - floral), and hedonic (1-4 = dislike - like). Samples were given randomly to avoid bias. A questionnaire sheet was given to the panelists as a medium to randomly provide an assessment of the product based on its code.

Statistical analysis

The parametric analysis of variance (ANOVA) test was used to assess data from chemical analyses. The SPSS 26.0 program performed parametric testing at a significance level of 0.05. If a treatment effect is

present, the Duncan Multiple Range Test (DMRT) is used to continue the test and determine the mean value of the difference. The non-parametric Kruskal-Wallis and Mann-Whitney tests were employed for data analysis on the sensory test.

Results and Discussion

The data in this study is an in-depth investigation of GTOC characteristics in comparison to other types of cookies on the market, including commercial GTOC and wheat cookies (Figure 1). This study examines cookies qualities based on nutritional data, functional components, and sensory value.

Nutritional facts

GTOC nutritional facts were determined through proximate values (Table 1) and total energy calculations (Figure 1). Based on the proximate analysis, GTOC nutritional facts were not significantly different from other cookies regarding moisture, ash, and protein (p>0.05). All cookies in this study used oatmeal and wheat flour as their primary raw materials. On average, they contained almost the same protein and ash content, namely 13% and 1.5–2% respectively (16). Cookie production generally involves a baking process with an average temperature of 180 °C for 30 minutes. The high cooking temperature in the production process of a food product will certainly produce dry food with an average water content of less than 8% (17).

The carbohydrate content of GTOC is significantly higher than wheat cookies, while the carbohydrate content of oatmeal cookies and commercial GTOC is not significantly different. The high carbohydrate content of oatmeal-based cookies is due to the type of complex carbohydrates that are the body's primary energy source, such as dietary fiber, which is both digestible and undigestible. This is in line with (18) that the fiber content of oats is much higher than wheat, namely 136 g/kg and 18 g/kg, respectively. On the other hand, the fat content of GTOC is significantly lower than that of wheat cookies and commercial GTOC.



Figure 1. The product appearance of green tea-fortified oatmeal cookies (GTOC) resulted in this study (C) compared to wheat cookies (A), oatmeal cookies (B), and GTOC commercial (D and E).

Parameters (%)	Wheat Cookies	Oatmeal Cookies	GTOC	Commercial GTOC-1	Commercial GTOC-2
Moisture	7.20±0.54	3.11±1.20	6.72±1.08	5.63±1.12	4.60±0.11
Ash	3.03±0.12	2.95±0.87	3.19±0.60	3.13±0.60	2.86±0.26
Protein	3.91±0.24	4.63±1.87	5.32±1.69	1.20±0.80	3.77±1.20
Carbohydrate	67.32±0.95ª	75.35 ± 1.15^{b}	72.20 ± 0.62^{b}	71.14 ± 0.22^{b}	70.54 ± 0.25^{b}
Fat	20.13±1.21ª	16.95 ± 0.98^{b}	18.65 ± 1.20^{b}	22.34±1.19 ^a	22.96 ± 0.10^{a}

Table 1. Proximate Value of GTOC compared to other cookies.

Data was expressed as mean±standard deviation. Different superscribes in the same line showed significant differences among the cookies category.

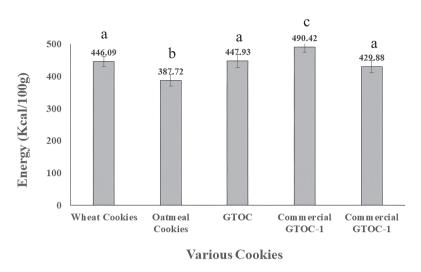


Figure 2. Energy total of GTOC compared to other cookies. Data was expressed as mean±standard deviation (error bars). Different letters above the bar graphs showed significant differences among the cookies category.

Table 2. Functional components of GTOC compared to other cookies.

Chemical Properties	Wheat Cookies	Oatmeal Cookies	GTOC	Commercial GTOC-1	Commercial GTOC-2
Catechin (mg/100 g)	ND	ND	24.77±1.62ª	10.00 ± 1.8^{b}	12.20±1.23 ^b
EGCG (mg/100 g)	ND	ND	12.50 ± 1.02^{a}	3.40 ± 0.45^{b}	4.60 ± 0.32^{b}
Antioxidant (%)	42.50±0.43ª	45.38±0.38ª	94.33 ± 0.69^{b}	52.50±1.2°	53.55±0.70°
β-Glucan (%)	0.75 ± 0.01^{a}	2.57 ± 0.23^{b}	$2.15\pm0.26^{\mathrm{b}}$	1.80 ± 0.19^{b}	2.11 ± 0.12^{b}

Data was expressed as mean±standard deviation. Different superscribes in the same line showed significant differences among the cookies category.

This is in line with research results (5), who found that the addition of GT powder can reduce the fat content in cookies due to the solid fat-binding activity of catechin in the dough. Catechin can form complex bonds with fat molecules, making extraction more difficult (19). The energy of a food product is measured to determine its value as a source of energy for the body, which is related to protein, carbohydrates, and fat (15). The amount of energy is shown in Figure 2. GTOC is known to have an energy value that is not significantly different from wheat cookies and commercial GTOC. This is related to comparing the values of protein, fat, and carbohydrates of the three, which are not significantly different (Table 1). The three nutritional components in the body are converted into energy. Every 1 gram of fat has an energy value of 9 calories, 1 gram of protein is worth four calories, and 1 gram of carbohydrate is worth four calories (20).

Functional component

Functional components are seen based on the content of catechin, epigallocatechin gallate (EGCG), antioxidants, and β -Glucan. The results of the analysis of catechin, EGCG, antioxidants, and β -Glucan can be seen in Table 2. In terms of functional components, wheat cookies, and oatmeal cookies did not contain catechin or EGCG, while GTOC contained catechin and EGCG with significantly higher concentrations than commercial GTOC.

Sensory Attribute	Wheat Cookies	Oatmeal Cookies	GTOC	Commercial GTOC-1	Commercial GTOC-2	Interpretation Scores (1-4)
Taste	3.40±1.06ª	3.22±0.88ª	3.05±1.11 ^b	2.50±0.27°	2.43±0.50°	Not sweet-sweet
Color	$1.80\pm0.88^{\circ}$	$2.02\pm0.08^{\text{b}}$	$3.88\pm0.02^{\circ}$	3.30 ± 0.25^{d}	3.39 ± 1.01^{d}	
						Not greenish-greenish
Aroma	2.35±0.75	2.50±0.77	2.80±0.25	2.42±0.88	2.69±0.27	Not floral-floral
Texture	2.50 ± 1.67^{a}	3.05 ± 1.55^{b}	3.11 ± 1.79^{b}	3.22 ± 0.94^{b}	3.28 ± 0.45^{b}	Not soft &chewy-soft and chewy
Hedonic	3.01 ± 0.77^{a}	2.89±0.55ª	3.78 ± 0.21^{b}	3.30±0.66ª	3.28 ± 0.78^{a}	Unfavored-favor

Table 3. Sensory Evaluation of GTOC compared to other cookies.

Data was expressed as mean±standard deviation. Different superscribes in the same line showed significant differences among the cookies category.

According to Table 2, a 6% addition of GTP in oat cookies contains more catechin and EGCG than GTC 1 and 2. The higher the catechin and EGCG content, the more GTP was added. GTC 1 and 2 were commercial cookies with an unknown percentage of GTP added. It indicates that the GTP content of the cookies was less than 6%. Green tea contains 20-45% polyphenols, 60-80% of which are catechins like epigallocatechin gallate (EGCG), known for their antioxidant, anti-inflammatory, and anti-carcinogenic properties (4). The antioxidant activity of 6% GTP added to oat cookies was also higher than that of any other product tested. Green tea's health benefits stem from the presence of natural antioxidants such as polyphenols, a diverse group of compounds that account for up to 30% of the dry weight of green tea (21). Polyphenols are considered extremely powerful antioxidants, with effects comparable to those of vitamins such as C and E, carotene, and tocopherol. According to studies, the high antioxidant potential of green tea stems from its high content of catechins, a type of phenolic compound with beneficial effects on human health (22). β-Glucan is a non-starch polysaccharide consisting of D-glucose molecules connected by β -1,3, β -1,4, and β -1,6 glycosidic chains (23). β -glucan is a water-soluble fiber found in almost all plant-based food products such as cereal products, mushrooms, etc (1). The β -Glucan values are shown in Table 2. The β -Glucan value of GTOC is significantly higher than wheat cookies but not significantly different from commercial GTOC. This is due to the difference in β -Glucan content in the main ingredients. The β -Glucan content of oats is higher than wheat. Oats

contain β -Glucan values of 2.2–7.8%, while wheat only contains β -Glucan of 0.4–1.4% (24).

Sensory value

Based on Table 3, the hedonic assessment with the most preferred scale is oat cookies with 6% green tea. The panelists obtained this from the assessment based on other attributes: taste, color, aroma, and texture of cookies. The taste at 6% GTOC has a sweet rating score from the panelists, even though the resulting sweetness is not as sweet as control cookies and wheat biscuits. Meanwhile, GTC 1 and 2 scored less sweet. Some panelists like cookies with a dominant sweet but not too sweet. In 6% GTP, GTC 1, and 2 cookies, the dominant sweet taste is slightly bitter. This is reasonable because of the presence of green tea, which has a bitter aftertaste, but this can be minimized by adding sugar or other ingredients so that theitter taste is not too flavorful (25). Meanwhile, in terms of color, the color at GTOC 6% is the most attractive. This is because the green tea put into the mixture gives a bright green color to enhance the appearance. While control one and wheat biscuits are the least attractive because the resulting color is pale. GTC 1 and 2 are almost the same color as GTOC 6% but darker.

The most preferred texture on cookies is GTC 2 cookies, this is because the level of crispness is just right and in accordance with the panelists, which is not too soft and not too hard. Meanwhile, the cookies control was a bit sluggish, thus reducing the panelists' preferences. The texture at 6% GTOC is still at the crispy level but a little harder than GTC 1 and 2.

Conclusion

The 6% GTOC is proven to have higher catechin, EGCG, and antioxidant content than other cookies. GTOC is the most preferred by panelists. Based on the higher content of chemical properties and the highest level of panelist preference, GTOC is the best treatment, as it is highly antioxidant.

Acknowledgments: We would like to express our gratitude for the cooperation of our partners, which are the Nutrition and Feed Science Laboratory and BRIN, which have helped and accommodated all the analysis processes to get the results we wanted to achieve.

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g., consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

Authors Contribution: SS: concept, supervision, design, data collection and processing, critical review; NN and DHW: concept, analysis and intrepretation, critical review; AIS: data collection, concept, critical review; KN: methodology, writing manuscript, critical review; AK and YA: critical review, writing manuscript. All authors have read and agreed to the published version of the manuscript.

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