Sorghum (*Sorghum bicolor* L. Moench var. bioguma) Cookies (SoKis): Source of Antioxidant and Prebiotic

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ABSTRACT

Sorghum bicolor L. Moench, locally called "*cantel,*" is an underused local food in Indonesia, which contains resistant starch and has the potential to act as prebiotic. Several studies have also reported the role of its phytochemical compounds as antioxidants. Therefore, this study aims to determine the potential of sorghum as a source of antioxidants and prebiotic in cookies products. Sorghum was dried under various temperatures (40, 55, and 70 °C) for 4 h, ground into flour, and used in cookies production to replace wheat flour (WF) in various ratios of sorghum flour (SF):WF (0:100, 25:75, 50:50, 75:25, 100:0 % (w/w)). Phytochemical compounds were tested using the maceration method and qualitatively by assessing the color change and physical appearance of SF. Antioxidants activity was analyzed using the 1,1-diphenyl-2- picrylhydrazyl (DPPH) method, while nutritional content was determined with proximate analyses. The total plate count (TPC) method was carried out to assess the growth of *Bifidobacterium longum*, and organoleptic test (n = 30) was performed using 5 points-hedonic scale. SF with a drying temperature of 55˚C was used as an ingredient for making cookies based on its moisture content (4.24 ± 0.084) and antioxidant activity value (70.77 ± 2.91%). The analysis results showed the presence of flavonoid, alkaloid, tannin, and polyphenol compounds in SF. Cookies with SF:WF ration of 50:50 (w/w) (SoKis) showed the best acceptance based on organoleptic test compared to the other formulation with antioxidant activity of 36.18 ± 2.56%. In addition, soKis contained 2.715% water, 1.425% ash, 24.57% fat, 8.24% protein, 63.05% carbohydrate, 0.255% crude fiber and could support the growth of *B. longum* with a value of 2.46×10^8 CFU/mL. Based on these results, sorghum could be used and developed as a functional food ingredient.

Keywords: Antioxidant; cookies; prebiotic; sorghum

INTRODUCTION

Sorghum is an underused local food in Indonesia, which is considered to be an inferior commodity compared to other cereal groups, such as rice, maize, and wheat (Suarni, 2012). This commodity is typically used as animal feed but has recently gained attention for its potential to yield fermentable sugars, serving as a source of renewable energy and raw materials for grain ethanol production (Dahlberg, 2019 and Stutts & Vermerris, 2020). In addition, sorghum can be processed as food using various methods for human consumption, such as cooking (rice) or mixing with other dry cereals and legumes (Pontieri & Del Giudice, 2016). In response to evolving social trends and dietary lifestyles, there is a growing demand for sorghum with high quality and several health effects (Aruna & Visarada, 2019). This demand is consistent with the increasing interest in functional food. Several studies have also explored functional food regarding its beneficial qualities and effects on human health (Helen N. & Ozioma F., 2022). This category of food is natural or processed

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and typically contains biologically active compounds. Functional food includes meals containing minerals, vitamins, fatty acids, and dietary fiber, as well as those added with biologically active substances, including antioxidants and prebiotic (Monica & Ioan, 2019).

As a local natural ingredient, sorghum has the potential to be used as a functional food due to its antioxidant compounds and resistant starch (Ratnavathi & Komala, 2016; Dávila et al., 2019). In addition, it contains 57-80.6% total carbohydrates, 4.4-21.1% protein, 2.1-7.6% fat, 1-3.4% crude fiber, 55.6-75.2% starch, 1.3-3.5% total minerals (ash), as well as various types of phenolic compounds and tannins serving as antioxidants (Ratnavathi & Komala, 2016). Various studies have also reported the presence of bioactive compounds, such as antioxidants, including carotenoids, flavonoids, minerals, phenolic acids, and tannins (Chávez et al., 2017). These compounds have been demonstrated to influence gut microbiota abundance and composition by decreasing reactive oxygen species (ROS) production, activating antioxidant enzymes, and engaging signaling pathways (Jiang et al., 2021; Kang et al., 2021). Studies on cells and animals have also indicated that *Bifidobacterium longum* strains can manage oxidative stress by enhancing the body's antioxidant activity (Yao et al., 2021). As a result, antioxidant compounds show promise as prebiotics, which are substrates that selectively utilized by microorganisms of the host to provide various health benefits (Gibson et al., 2017).

In line with previous studies, the main carbohydrate in sorghum is starch, with the majority of starch granules being highly digestible (30.0-66.2%), while the remaining goes through rapid digestion (15.3-22.6%) and are resistant (16.7-43.2%) (Mkandawire et al., 2013). This resistant starch (RS) content can be prebiotic, where it is used by bacteria in the gut for growth. Gut microbiotas typically use RS through the fermentation process, where the component is converted into short-chain fatty acids (SCFA), such as propionate, butyrate, and acetate that act positively towards enhancing health effect (Dávila et al., 2019; Holscher, 2017). The production of butyrate and propionate from hexose sugars is linked to various bacterial groups, though some species can also produce propionate from deoxy-sugars and lactate through different pathways. Additionally, lactate, which many gut bacteria produce in isolation, can be utilized by specific Firmicutes to generate butyrate, and its consumption plays a crucial role in sustaining a stable microbial community (Flint et al., 2015).

According to previous reports, some food processing techniques of sorghum include milling, flaking, extrusion, puffing, popping, and baking (Dayakar Rao et al., 2016). Sorghum in various countries is often used to replace or combined with wheat flour (WF) to produce bakery products, such as leavened or unleavened bread (Dendy, 1992), pasta (Benhur et al., 2015), cookies (Dayakar Rao et al., 2016), and other products. In addition, it can also be consumed in its whole form or processed into flour and combined with wheat to make products or snacks (Aruna & Visarada, 2019), such as cookies. Apart from its high popularity, the cost used in the manufacturing process is also relatively low and has a high shelf-life stability, making it a potential means of increasing the nutritional enrichment of the community. A study by Olurin et al. (2020) found that substituting WF with sorghum flour (SF) improved nutrition in cookies. Therefore, this study aims to determine the potential of sorghum as a source of antioxidants and prebiotic in pastry products as a functional food.

METHODS

Materials

White sorghum (*Sorghum bicolor* L. Moench var. bioguma) (Krya brand) was purchased from Pasar Beringharjo, Yogyakarta, and *Bifidobacterium longum* isolate (FNCC 0463) was obtained by purchasing the isolate from Pusat Studi Pangan dan Gizi, Universitas Gadjah Mada, Yogyakarta. All chemicals and reagents were analytical grades, while ingredients for cookies production were food grade.

The equipment used includes an oven (Memmert UN55, Germany), a UV-Vis spectrometer (Genesys 10S UV-Vis, China), a cabinet dryer (Gama Mesin Mandiri, Indonesia), a water bath (Memmert, Germany), and a centrifuge (OHAUS, USA).

SF Preparation

Sorghum was dried using a cabinet dryer for 4 h under various temperatures (40, 55, 70 °C), ground, and sieved (100 mesh) to produce SF, then stored in zip plastic. Yield of SF was calculated and SF from each drying temperature was measured for its moisture content according to Equation 1 (AOAC, 2007).

$$\%\ Moisture\ Content = \frac{b-(c-\alpha)}{b} x\ 100\% \quad (1)$$

Description:
b = weight of the sample before drying
c = weight of porcelain cup + sample drying result
a = initial porcelain cup weight

Phytochemical & Iodine Test

SF was soaked into 70% ethanol for 72 h to obtain SF extract and was tested qualitatively for its

phytochemical content on flavonoids, saponin, alkaloids, steroid-terpenoids, tannins, and polyphenols (Agustina et al., 2021). An iodine test was carried out by adding an iodine solution to SF to determine the presence of carbohydrates contained in SF (Fitri & Fitriana, 2020).

Sorghum Cookies Production

The production of cookies referred to the procedure from Adeyeye, (2016) & Sustriawan et al. (2021) with modifications. The first step was to make the dough by mixing the ingredients (w/w) of SF & WF (0:100, 25:50, 50:50, 75:25, and 100:0, respectively), sugar (38%), butter (50%), salt (0.9%), egg yolk (1 piece), and (0.1%) baking powder, and this was formed into a flat round and baked at 140˚C for 25 min, and cookies were finished and ready for further analysis.

Antioxidant Activity Test Using 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Method

Antioxidant activity test was conducted on SF and cookies using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical capture method. Samples (0.1 g) were added with distilled water (10 mL), and centrifuged (6.000 RPM) for 10 min. Five concentrations of supernatant (10,000 ppm, 8,000 ppm, 6,000 ppm, 4,000 ppm, and 2,000 ppm) were then formed, and each concentration (1 ml) was added with 2 ml of 0.1 mM DPPH solution (1:2; v/v), incubated at room temperature (25-28 °C) under dark conditions for 30 min. The solution was then measured for its absorbance at 517 of a wavelength using a UV-Vis and was prepared with the same procedure which only contained distilled water and DPPH solution. Antioxidant activity was then calculated by Equation 2 from Pangestu et al. (2017) with modification.

$$\%\ Antioxidant\ activity = \frac{control\ absorbance - sample\ absorbance}{control\ absorbance} x\ 100\% \quad (2)$$

Bacterial Enumeration Using Total Plate Count (TPC) Method

Using de Man Rogossa Sharpe (MRS) broth media, *Bifidobacterium longum* FNCC 0463 stock was inoculated into the media and incubated for 24-48 hours at 37 °C until white bacterial colonies formed at the bottom of the test tube. Bacterial colonies were inoculated into MRS agar medium and incubated for 24 hours at 37 °C under anaerobic conditions (Amer et al., 2014). The result of incubation formed growing bacterial colonies characterized by the formation of round milky white colonies uniformly without any mucus around them. Bacterial dilution was carried out by inserting 1.000 µL (1 mL) of re-culture result into the first dilution tube (10^{-1}) containing 9 mL of peptone solution and then homogenized, and 1.000 µL (1 mL) was taken back to be inserted into the second dilution tube (10^{-2}). The step was repeated until the 10^{-7} dilution series, and this was done to determine the optimum dilution that could grow bacteria in the range of 30-300 CFU/mL. TPC method was carried out by replacing the carbon source of the media (MRS agar), namely dextrose with SF & cookies, and this was dissolved first with distilled water and then centrifuged, and the supernatant was taken and mixed with other media ingredient. For cookies' product, cookies were first ground then dissolved with distilled water, centrifuged, and the supernatant was taken and mixed with other media ingredients. The media (150 mL) contained 1.5 g beef extract, 0.75 g sodium acetate, 1.5 g peptone, 0.3 g ammonium citrate, 0.75 g yeast extract, 0.26 g dipotassium phosphate, 0.0075 g manganese sulfate, and 0.015 g magnesium sulfate. Furthermore, 0.15 mL of Tween 80 solution, 3 g sample, and 1.8 g agar were added, and all ingredients were then dissolved with distilled water to reach a volume of 150 mL and sterilized using an autoclave. Bacterial inoculation on MRS agar media was carried out using the pour plate technique by pouring 100 µL of bacterial solution from the dilution series into a sterile Petri dish and covered with 15 mL of MRS agar media, homogenized, then incubated for 24 hours (37 °C). Colony counts are carried out with a colony count range of 30-300 colonies per petri dish, if less than 30 colonies are considered too few to represent the sample, and if more than 300 colonies are considered Too Many to Count (TNTC). The number of colonies (CFU/mL) was calculated with Equation 3 (Azizah & Soesetyaningsih, 2020.)

$$\frac{CFU}{mL} = \sum colony\ per\ petri\ x \frac{1}{dillution\ factor} \quad (3)$$

Organoleptic Test

Organoleptic test of cookies product used 5-point hedonic scale method on 30 panelists of the Department of Biology students, Faculty of Biotechnology, Universitas Kristen Duta Wacana which aged from 19-24 years with assessment attributed including color, aroma, texture, and taste with a numerical scale of very dislike (1), dislike (2), ordinary (3), like (4), and very like (5) (Farrah et al., 2022).

Proximate Analyses

The nutritional content of the product was carried out to determine the content of protein (Kjeldahl method), ash (Gravimetry method), water (Gravimetry method), fat (Soxhlet method), crude fiber (Gravimetry method), and carbohydrates (AOAC, 2007).

Statistical Test

The data obtained were statistically analyzed using the SPSS software version 25 for Windows (IBM Corp. New York, USA). One-way ANOVA was conducted on antioxidant activity test and organoleptic test was measured using Duncan's multiple range test ($p \leq 0.05$) to study differences between means for color, aroma, texture, taste, and overall acceptability.

RESULTS AND DISCUSSION

SF Characteristics Moisture Content

Moisture content in SF was measured during 4 h of drying time and presented in Table 1.

Table 1. The moisture content of SF with different drying temperatures

Sample	Drying temperature (°C)	Moisture content (%)
SF	40	2.00 ± 0.132
	55	4.24 ± 0.084
	70	5.53 ± 0.381

Table 1 showed that SF with drying temperatures of 40 °C, 55 °C, and 70 °C contained an average moisture of 2%, 4.24%, and 5.53% (w/w), respectively. All SF produced in this study met the quality requirement for flour moisture content (max. 14%) based on Indonesia National Standard (SNI 01-3157 (1992)). The lower the moisture content contained in the flour, the longer the shelf life of the flour (Budiarti, Sulistiawati, et al., 2021). In this study, the higher the drying temperature used, the higher the value of moisture content in SF. This could be caused by several factors including poor storage, material surface area, and the thickness of the dried material (Surahman & Sofyan, 2017). The higher the temperature and the longer the drying time used to dry a material, the more water evaporated (Budiarti, Sya'bani, et al., 2021.

Carbohydrate Content

In this study, a qualitative analysis of carbohydrates was carried out by giving a reaction with iodine solution on SF which aimed to determine that SF had carbohydrate content, namely starch, where this starch had potential as prebiotic (Al-Sheraji et al., 2013). The results of the qualitative analysis of carbohydrates in SF were presented in Table 2.

Table 2. Qualitative analysis result of sorghum starch

Sample	Result	Description
SF 40 °C	+	Blue to purple-black
SF 55 °C	+	Blue to purple-black
SF 70 °C	+	Blue to purple-black

The result of the qualitative analysis of starch in Table 2, all samples showed positive results, which were showed by a change in color to blue-bronze-black. This showed that SF contained carbohydrates, and this was reinforced by the statement of (Mkandawire et al., 2013), that the main carbohydrate contained in sorghum was starch, where most of the starch granules could be digested thoroughly (30.0-66.2%) (Sang et al., 2008), while the rest was digested quickly (15.3-22.6%), and resistant (16.7-43.2%). The starch content contained in SF had potential as prebiotic (Al-Sheraji et al., 2013).

Antioxidant Activity

Antioxidant activity of SF was presented in Table 3.

Table 3. Antioxidant activity of SF

Sample	Antioxidant activity (%)
Vitamin C (Control)	59.08 ± 4.310^{C}
SF 40 °C	64.85 ± 8.700^{C}
SF 55 °C	70.77 ± 2.910^{C}
SF 70 °C	70.44 ± 8.00^{C}

Notes: SF = SF, mean values with different superscripts in the same column showed significant Differences ($p \leq 0.05$).

The results of antioxidant activity test in Table 4.3 showed that antioxidant activity of the TS 40 °C, TS 55 °C, and TS 70 °C samples did not have a significant difference with the control, namely vitamin C. In the analysis of antioxidant activity in raw materials, namely SF (TS 40 °C, TS 55 °C, and TS 70 °C), SF with a drying temperature of 55 °C had the highest antioxidant activity of 70.77%. Therefore, SF with a drying temperature of 55 °C was chosen as an ingredient for making sorghum cookies, which was also based on the moisture content of flour that met SNI 01-3157. In Table 3, there was an increase in antioxidant activity as the drying temperature increased, it was suspected that during maceration, antioxidant compounds contained in sorghum were extracted unevenly. Nisa (2010) stated that different levels of polarity caused the extracted

antioxidant compounds to be different. Furthermore, the amount of antioxidant activity produced was also different.

Phytochemical Compounds

The identification of phytochemical compounds was carried out to see the chemical compounds contained in SF, and some chemical compounds that could be identified were flavonoids, saponins, alkaloids, steroid-terpenoids, tannins, and polyphenols. The results of the identification of phytochemical compounds were presented in Table 4.

Table 4. Identification result of phytochemical compounds in SF

Sample	Chemical compounds	Result
SF 55 °C	Flavonoid	+
	Saponin	-
	Alkaloid	+
	Steroid & terpenoid	-
	Tanin & polifenol	+

Based on the phytochemical test presented in Table 4, it was found that SF contained flavonoid, alkaloid, and tannin compounds as showed by positive (+) results, and the results of flavonoid compounds were showed by a change in color to orange. Positive results of alkaloid compounds were showed by the presence of white, light brown, and red-orange precipitates. Positive results for tannin and polyphenol compounds were showed by the presence of a bluish-black precipitate (Agustina et al., 2021). The study results from the phytochemical test showed that there were chemical compounds contained in SF such as flavonoids, tannins, and polyphenols, which had antioxidant activity abilities. This was reinforced by the statement (Shahidi & Zhong, 2015) that antioxidants were naturally available in plants, specifically higher plants that were rich in providing natural sources of antioxidants, such as tocopherols and polyphenols which were found in spices, herbs, fruit, vegetables, cereals, grains, seeds, tea, and oils. Chavez et al. (2017) reported that bioactive compounds from the group of carotenoids, flavonoids, minerals, phenolic acids, and tannins contained in sorghum had the potential to be used as a source of antioxidants.

Acceptability And Antioxidant Activity of Sorghum Cookies

Acceptability of sorghum cookies

Cookies were made by substituting the main ingredient, WF, with SF various variations and organoleptic properties were color, aroma, texture, and flavor. Table 5 presented the result of the organoleptic test to see the best cookies acceptance from the hedonic method.

Based on Table 5, for the organoleptic test of all parameters, the substitution levels of 50% (cookies C), 75% (cookies D), and 100% (cookies E) were not significantly different from each other when compared to cookies A (control) and cookies B, and it was significantly different from 100% WF. However, it was significantly different from 100% WF (control) in color and texture parameters, and not significantly different in aroma and taste parameters except for cookie E in the aroma parameter which was significantly different from cookies A (control). A significant difference between cookies A and sorghum cookies was seen in the color parameter, and this was because the higher the concentration of SF in cookies, the darker the color of cookies. The effect was similar for sorghum-blended cookies, where the cookies became darker with increasing SF blend concentration in the study by Awobusuyi et al. (2020).

Table 5. Organoleptic test result of sorghum cookies

Sample code	Wheat flour : sorghum flour	Color	Aroma	Texture	Flavor	Average acceptance
Cookies A	100:0	4.40 ± 0.712^{b}	4.20 ± 0.945^{c}	4.30 ± 0.690^{b}	4.37 ± 0.836^{c}	4.32 ± 0.0769^{c}
Cookies B	75:25	3.70 ± 1.005^{a}	3.47 ± 1.176^{a}	3.67 ± 0.978^{a}	2.70 ± 1.320^{a}	3.38 ± 0.405^{a}
Cookies C	50:50	3.80 ± 0.945^{a}	4.07 ± 0.772^{c}	3.87 ± 0.957^{a}	3.90 ± 0.597^{bc}	3.91 ± 0.099^{b}
Cookies D	25:75	3.70 ± 0.936^{a}	3.97 ± 0.752^{bc}	3.77 ± 1.023^{a}	4.07 ± 0.929^{bc}	3.87 ± 0.148^{b}
Cookies E	0:100	3.77 ± 0.883^{a}	3.63 ± 1.048^{ab}	3.57 ± 0.989^{a}	3.83 ± 1.035^{b}	3.70 ± 0.104^{bc}

Notes: n = 30, mean values with different superscripts in the same column showed significant differences ($p \leq 0.05$)

Based on the organoleptic results, in the aroma and texture parameters, most panelists could still accept cookies that were substituted with SF up to 50%. This was in line with the statement of (Pontieri & Del Giudice, 2016) which stated that the use of SF in bakery products could substitute the main ingredient, namely WF from levels of 5% to 50%. The result showed that cookies with the best acceptance besides the control were cookies C (50%WF:50%SF). Cookies C (SoKis) with a composition of 50% WF, and 50% SF was cookies with the best acceptance, then it could be tested for prebiotic potency, antioxidant activity and nutritional content.

Antioxidant activity of sorghum cookies

Measurement of antioxidant activity in this study used the DPPH method, by looking at the percentage of sample inhibition in counteracting free radicals. Table 6 presented the result of measuring antioxidant activity of Vitamin C (control), raw materials (SF), and sorghum cookies products. Antioxidants were substances that, when present in food or the body, could delay, control, or prevent oxidative processes. These antioxidant compounds were needed by the body to delay or even prevent oxidative damage by stabilizing free radicals (Shalaby, 2019).

Table 6. Antioxidant activity measurement result

Sample code	WF:SF	Antioxidant activity (%)
Vitamin C (control)	-	59.08 ± 4.31^{c}
Cookies A	100:0	13.48 ± 4.56^{a}
Cookies B	75:25	34.75 ± 1.30^{b}
Cookies C	50:50	36.18 ± 2.56^{b}
Cookies D	25:75	31.74 ± 6.26^{b}
Cookies E	0:100	26.53 ± 2.92^{b}

Notes: Mean values with different superscripts in the same column showed significant differences ($p \leq 0.05$).

The results of antioxidant activity test in Table 4.6 showed that antioxidant activity of cookies products B, C, D, and E had a significant difference with the control, while cookie A had antioxidant activity that was significantly different from other cookies and with the control. Based on the results of antioxidant activity test presented in Table 4.6, it was also found that cookies C had a higher antioxidant activity value of 36.18% compared to cookies A (100%TT:0%TS) (control) but did not have a significant difference to cookies B, D, and E. Antioxidant activity test results showed that substituting WF with SF could increase antioxidant activity of the product, but it could also decrease with a higher concentration of substitution. The decrease in antioxidant activity value began to occur at 75% and 100% SF substitution (Table 6). Priftis et al. (2015) reported that some compounds synthesized during roasting such as melanoidin could contribute to the increase in antioxidant activity. However, in the preparation of sorghum cookies, not all of them were baked at the same time, and because of this, more time was needed in the baking process. Further heating or the use of higher temperatures could decrease antioxidant activity of the product, and this was due to compositional degradation and other reactions such as the degradation of phenolic compounds and their binding to proteins that inhibited their ability to react with free radicals, as reported by a previous study (Kamiyama et al., 2015).

Prebiotic potency of sorghum cookies

Prebiotic potential analysis was conducted for SF 55 °C and cookies C, which aims to determine the ability of raw materials and products to support the growth of *B. longum*, and the results are presented in Table 7.

Table 7. Bacterial population (CFU/mL) from the sample

Sample	Bacterial Population (CFU/mL)
Control (D-glucose)	2.80×10^{8a}
SF 55 °C	2.50×10^{8a}
Cookies C	2.46×10^{8a}

Based on the results shown in Table 7, SF 55 °C and cookies C showed the same ability to support *B. longum* growth as the control, where there was no significant difference (One-Way ANOVA, $p \leq 0.05$), and this study did not conduct experiments to test the type of carbohydrates in sorghum. Based on the studies conducted by (Mkandawire et al., 2013), sorghum contained resistant starch which acted as prebiotic, and previous studies had reported that resistant starch could modulate the composition of the gut microbiota by producing SCFA (Gargari et al., 2015; Kleessen et al., 1997; Paturi et al., 2012; Ren et al., 2022). The studies conducted by (Kleessen et al., 1997) found that resistant starch given to mice increased SCFA production and increased the population of *Bifidobacterium* spp in the mouse intestine. Further reports (Martínez et al., 2010) conducted in humans resistant starch diet was shown in humans to increase SCFA. In addition to resistant starch, antioxidant compounds contained in sorghum could also affect the abundance of gut microbiota, and this

Table 8. Results of nutrient content analysis on SoKis

Parameters	SNI No. 01-2973-1992	Cookies C
Water (%)	Max. 5	2.175
Ash (%)	Max. 1.5	1.425
Fat (%)	Min. 9.5	24.57
Protein (%)	Min. 5	8.24
Carbohydrates (%)	Min. 70	63.05
Crude fiber (%)	Max. 0.5	0.255
Calories (Calories/100 g)	Min. 400	506.29

increased antioxidant activity changes the abundance and composition of the gut microbiota, which in turn reduced the production of ROS. Experimental cell and animal studies had shown that *B. longum* strains regulated oxidative stress by increasing the body's antioxidant activity (Yao et al., 2021).

Nutritional Content of Sorghum Cookies

Nutritional content analysis was carried out on SoKis (50WF:50SF, w/w) with the highest acceptance based on organoleptic tests and antioxidant activity values. The analysis of nutritional content was carried out with a proximate test which included tests of water content, ash, fat, protein, carbohydrates, and crude fiber. The results of the nutritional content analysis on SoKis were presented in Table 8.

The results of the nutritional content analysis of SoKis in Table 8 showed that SoKis contained water content of 2.715%, ash content of 1.425%, fat content of 24.57%, protein content of 8.24%, carbohydrate content of 63.05%, and crude fiber content of 0.255%. Carbohydrates, fats, and proteins served as a source of calories that the body used and these 3 macronutrients usually covered all calories in food. The higher the calorie content in food, the more energy it can supply to the body. This energy is released when the body digests and absorbs the food (FDA, 2020). Compared to the quality requirements of cookies based on SNI 01-2973-1992, all parameters of the nutritional content of cookies C had met the quality requirements, except for the carbohydrate content, where the carbohydrate content contained in cookies C had not reached the minimum limit of quality requirements. This low carbohydrate could be influenced by several factors including high roasting temperature (Risnoyatiningsih et al., 2011), the lower carbohydrate content of sorghum than wheat (Rahmawati & Wahyani, 2021), and the tannin content in sorghum which binds to carbohydrates, causing the carbohydrate content to decrease (Aprilia, 2015). Based on the study results (Table 4.8), cookies C could be said to have met the quality requirements of cookies.

CONCLUSION

In conclusion, sorghum could be used as a substitute for WF in cookies products. The sample containing 50% SF showed antioxidant activity of 36.18 ± 2.56% and could support the growth of *B. longum* 2,46 × 10^8 CFU/mL for 24 h fermentation. Therefore, study sorghum could be used and developed as a functional food ingredient. Further experiments were needed to assess the type of resistant starch contained in sorghum that could support the growth of *B. longum.*

CONFLICT OF INTEREST

There was no conflict of interest to be declared.

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