Food Sci. Preserv., 32(5), 823-835 (2025)
pISSN: 3022-5477, eISSN: 3022-5485

Food Science and Preservation
https://doi.org/10.11002/fsp.2025.32.5.823

Research Article

Quality characteristics and antioxidant properties of cookies fortified with brewer's spent grain powder

Jisung Cheung, Eun-Sun Hwang*

Major in Food and Nutrition, School of Wellness Industry Convergence, Hankyong National University, Anseong 17579, Korea

Abstract This study investigated the effects of adding brewer's spent grain (BSG) powder at varying concentrations (10-40%) on the quality characteristics and antioxidant activity of cookies. Physicochemical analysis revealed that as BSG content increased, cookies' spread factor, leavening rate, moisture content, pH, lightness, and yellowness significantly decreased. Conversely, hardness, ash content, protein content, acidity, soluble solids, redness, and browning index showed marked increases. The total polyphenol and flavonoid contents in cookies increased proportionally with BSG addition, reaching 4.26-fold and 4.05-fold higher values than those of the control, respectively. Correspondingly, antioxidant activities, as measured by ABTS radical scavenging and reducing power assays, were significantly enhanced with high BSG concentrations, whereas DPPH radical scavenging showed a modest increase. These results suggest that BSG powder can serve as a valuable functional ingredient in cookie formulations, improving antioxidant properties and affecting the physical and sensory attributes. This study highlights the potential of upcycling food industry byproducts into health-promoting baked goods, contributing to sustainable food processing and functional food development.

Keywords brewer's spent grain, cookies, antioxidant activity, dietary fiber

Citation: Cheung J, Hwang ES. Quality characteristics and antioxidant properties of cookies fortified with brewer's spent grain powder. Food Sci. Preserv., 32(5), 823-835 (2025)

Received: July 23, 2025
Revised: August 20, 2025
Accepted: August 20, 2025

***Corresponding author**
Eun-Sun Hwang
Tel: +82-31-670-5182
E-mail: ehwang@hknu.ac.kr

1. Introduction

Brewer's spent grain (BSG), which accounts for approximately 85% of the total solid waste produced during brewing, is one of the most abundant byproducts in the beer industry, with tens of millions of tons generated globally each year (Aradwad et al., 2025; Saberian et al., 2024). BSG is typically utilized as animal feed or compost, or is otherwise discarded, which can lead to disposal costs and environmental pollution (Aradwad et al., 2025; Merten et al., 2022; Saberian et al., 2024). BSG primarily consists of malt husks and residual proteins and has been reported to contain 15-26% protein and 35-60% dietary fiber on a dry weight basis, which is higher than that of other cereal by-products (Lynch et al., 2016; Shen et al., 2019). In particular, the non-cellulosic polysaccharides in BSG act as prebiotics that promote the growth of beneficial intestinal bacteria, thereby contributing to improved gut health and the prevention of constipation (MinAlexander et al., 2023; Nyhan et al., 2023). Moreover, the dietary fiber in BSG slows the rate of digestion and helps regulate postprandial blood glucose levels (Nyhan et al., 2023). BSG is also rich in polyphenolic compounds, such as phenolic acids, which exhibit strong antioxidant activity by scavenging reactive oxygen species and preventing cellular damage (Ikram et al., 2017; Virdi et al., 2025). Among these, ferulic and p-coumaric acids are the major phenolic acids present in BSG (Ikram et al., 2017). These antioxidant components may also play a role in delaying aging and preventing chronic diseases (Virdi et al., 2025). Furthermore, BSG contains proteins with a high glutamine content, suggesting its potential as a plant-based protein source, particularly for vegetarians or consumers who prefer plant-based diets. BSG can be standardized through drying and milling for consistent quality. Its incorporation into bakery production is feasible with minimal adjustments to existing processes, making large-scale application practical (MinAlexander et al., 2023; Perez-Alva et al., 2025). From a market perspective, BSG-enriched products meet the rising demand for sustainable and functional foods and can be commercialized through collaborations between breweries and bakeries, development of health-oriented bakery lines, and expansion into mainstream categories such as cookies and bread (Nicolai et al., 2025; Nyhan et al., 2023; Saberian et al., 2024).

Owing to these nutritional and functional properties, BSG has recently gained attention as a promising ingredient for food upcycling. In recent years, research and product development using BSG in food upcycling have been actively pursued, especially in countries outside Korea (Nyhan et al., 2023). Incorporation of BSG into pasta has been shown to significantly increase the contents of dietary fiber, resistant starch, and β-glucan. Additionally, various bakery and processed food products, such as bread, cereals, and crackers, have been developed using BSG to enhance their nutritional value and functional properties (Merten et al., 2022; Nicolai et al., 2025; Saberian et al., 2024). These efforts support the notion that BSG should no longer be viewed as a mere industrial byproduct, but rather as a valuable resource for the food industry’s future.

Cookies are one of the most popular bakery products worldwide. It is typically made from wheat flour, sugar, and fats and is enjoyed by people of all ages (Joo and Choi, 2012). Cookies are convenient to consume and offers wide variability in shape and flavor, making it a versatile confectionery item that caters to diverse consumer preferences. However, conventional cookies often contain high levels of sugar and fat, which may result in a poor nutritional balance (Young et al., 2016). To address this issue and meet the growing demand for health-promoting foods, recent studies have focused on fortifying cookies with functional ingredients to enhance their nutritional value and functionality. Studies have reported the use of various food byproducts rich in functional compounds, such as apple pomace (Oh and Kang, 2016), citrus peel (Chio, 2021), onion skin (Yeom and Hwang, 2020), and spinach powder (Lee, 2025), to improve the antioxidant content, vitamin and mineral levels, and visual appeal of cookies. However, there are limited studies on the quality and functionality of cookies supplemented with BSG. Previous findings have suggested that the addition of functional ingredients can improve both the nutritional and functional properties of cookies; however, the level of supplementation may affect physical characteristics (e.g., hardness, spread factor, and color) and sensory qualities (e.g., taste, aroma, and texture), highlighting the need to determine the optimal substitution ratio.

This study aimed to evaluate the quality characteristics and functionality of cookies fortified with BSG powder, based on the nutritional superiority of BSG, and within the context of food upcycling. As cookies are widely consumed snack products, the incorporation of BSG powder is expected to enhance their nutritional value and provide novel textural and flavor attributes. Through this approach, this study seeks to expand the applicability of BSG as a functional food ingredient and contribute to the development of a more sustainable food system.

2. Materials and methods

2.1. Materials and chemicals

Cake flour and baking powder (Samyang Co. Ltd., Asan, Korea), butter (Seoul Milk, Yangju, Korea), eggs (CJ Freshway, Icheon, Korea), and granulated sugar (CJ Cheiljedang Co. Ltd., Incheon, Korea) used for cookie preparation were purchased from local markets. BSG was provided by a craft brewery located in Seoul, Korea. Ethanol, ethyl ether, and 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Fluka (Buchs, Switzerland). Gallic acid, catechin, Folin-Ciocalteu reagent, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All the other reagents were purchased from Junsei Chemical Co., Ltd. (Tokyo, Japan).

2.2. Preparation of BSG powder

BSG was obtained from a local craft brewery in Seoul, Korea, after completion of the beer brewing process. The collected BSG was washed twice with running water, manually squeezed to remove excess moisture, and drained through a sieve. The drained BSG was placed in aluminum trays, pre-frozen at -80℃, and then rapidly frozen and

https://doi.org/10.11002/fsp.2025.32.5.823

lyophilized using a freeze dryer (FDU-1200, EYELA, Tokyo, Japan). The freeze-dried BSG was ground three times for 1 min using a high-speed grinder (PGR 002M, Supreme Electric Co. Ltd., Korea). The ground material was sieved to obtain powder with a particle size less than 850 μm (standard sieve No. 20). The final BSG powder was stored at -20℃ until further use.

2.3. Preparation of BSG-fortified cookies

The cookie formulation was determined based on a previous study (Yeom and Hwang, 2020) and several preliminary experiments (Table 1). To maximize the functional benefits of freeze-dried BSG powder, the highest possible substitution level was determined. Preliminary trials showed that when the BSG powder exceeded 40% of the wheat flour weight, the dough became excessively dry and rough in texture. Therefore, the maximum substitution level was set to 40%. The control group was prepared without BSG powder, whereas the experimental groups were formulated by replacing 10%, 20%, 30%, and 40% of the wheat flour with BSG powder. The butter was softened at room temperature and creamed with gradually added sugar using a hand mixer. The eggs were added in three portions and mixed thoroughly to prevent phase separation. Sifted wheat flour, baking powder, and an appropriate amount of BSG powder for each experimental group were added, and the dough was mixed using a rubber spatula until uniform. The dough was shaped into 4×4 cm squares using a plastic wrap and rested in a refrigerator for 3 h. After resting, the dough was cut into 0.5 cm thick slices, placed on an oven tray, and baked for 15 min in a preheated oven (FDO-7104B, Daeyoung Bakery Machinery Inc., Seoul, Korea) at 160℃ (top heat) and 140℃ (bottom heat). The baked cookies were cooled to room temperature for 30 min prior to analysis.

2.4. Measurement of spread factor, leavening rate, and baking loss rate

The spread factor, leavening rate, and baking loss rate of cookies were measured according to the methods described by the American Association of Cereal Chemists (AACC, 2000). For the spread factor, six cookies were aligned horizontally in a row, and the total length was measured. After rotation by 90°, the total length was measured using the same method. The average of the two measurements was used to calculate the average diameter. Thickness was determined by vertically stacking six cookies, measuring the total height, rearranging the stacking order, and repeating the measurements. The average of these two values was used as the final thickness.

The leavening rate was calculated based on the difference in cookie weight before and after baking, using the following equation:

Leavening rate (%)
= (Weight difference before and after baking of experimental groups / Weight difference before and after baking of control group) × 100

Table 1. Formula for cookies fortified with brewer's spent grain powder

Ingredients (g)	Brewer's spent-grain powder (%)[1)]				
	0	10	20	30	40
Brewer's spent-grain powder	0	12	24	36	48
Wheat flour	120	108	96	84	72
Butter	42	42	42	42	42
Sugar	30	30	30	30	30
Egg yolk	28.8	28.8	28.8	28.8	28.8
Baking powder	0.6	0.6	0.6	0.6	0.6
Salt	0.6	0.6	0.6	0.6	0.6
Total	222	222	222	222	222

[1)]Brewer's spent-grain powder (10, 20, 30, and 40%) was added based on the total weight of wheat flour.

The baking loss rate was calculated using the weight loss before and after baking as follows:

Baking loss rate (%)
= [(Weight before baking - Weight after baking) / Weight before baking] × 100

2.5. Proximate composition analysis

The cookies were ground into a uniform powder using a grinder (PGR 002M, Supreme Electric Co. Ltd., Guangzhou, China) prior to analysis of the proximate composition following the AOAC official methods (AOAC, 1995). Moisture content was determined by drying the samples at 105℃ using a dry oven (EYELA, Tokyo, Japan). Crude protein content was determined using the semimicro-Kjeldahl method with a protein analyzer (Kjeltec 2400 AUT, Foss Tecator, Sweden). Crude fat content was measured using a Soxhlet extractor (Soxtec System HT 1043, Foss Tecator, Sweden) after extraction with diethyl ether. Crude ash content was measured after incineration of the samples at 600℃ in a muffle furnace (SH-FU-11MGE, Samheung Scientific, Sejong, Korea).

2.6. Measurement of soluble solids, pH, and hardness

Cookies prepared using varying concentrations of BSG powder were ground into a uniform powder using a grinder (PGR 002M, Supreme Electric Co. Ltd., Guangzhou, China). Five grams of each powdered sample was mixed with 10 mL of 95% ethanol, and the mixture was subjected to ultrasonic extraction at 40℃ for 10 min. The extract was centrifuged at 13,500 ×*g* for 30 min (Mega 17R, Hanil Co., Daejeon, Korea). The supernatant was used for measuring soluble solid content using a refractometer (PR-201α, Atago Co.,Tokyo, Japan) and pH using a pH meter (S400, Mettler-Toledo, Columbus, OH, USA).

Texture profile analysis (TPA) was conducted at room temperature on the day of cookie preparation to measure the hardness using a texture analyzer (CT3 10 K, Brookfield, Middleboro, MA, USA). A TA25/1000 probe was employed, and the analysis was performed under the following conditions: target type set to % deformation, target value of 60%, pre-test speed of 2.0 mm/sec, test speed of 10.0 mm/sec, and load cell of 100 g.

2.7. Measurement of color and browning index (BI)

The color of the cookies was measured using a colorimeter (ChromaMeter CR-300; Minolta, Tokyo, Japan) to determine the lightness (L*), redness (a*), and yellowness (b*) values. The standard white calibration plates had L*, a*, and b* values of 97.10, +0.24, and +1.75, respectively.

The BI, which quantitatively evaluates the degree of non-enzymatic browning in cookies, was calculated based on the color values using the following equation (Hwang and Kim, 2024):

$$BI = 100\ (x - 0.31)\ /\ 0.17$$

where, $x = (a^*_{sample} + 1.75\ L^*_{sample})\ /\ (5.645\ L^*_{sample} + a^*_{sample} - 3.012b^*_{sample})$

2.8. Determination of total polyphenol and total flavonoid contents

The powdered cookies (5 g) were mixed with 20 mL of 95% ethanol using a vortex mixer for 15 s to achieve homogeneity, followed by centrifugation at 13,500 ×*g* for 10 min. The resulting supernatants were used for the analysis of total polyphenols and flavonoids.

For total polyphenol determination, an appropriately diluted sample extract (0.5 mL) was mixed with 2 N Folin-Ciocalteu reagent and reacted at room temperature for 3 min. Subsequently, 1.5 mL of 2% sodium carbonate solution was added, and the mixture was incubated in the dark for 2 h. The absorbance of the reaction mixture was measured at 760 nm using a spectrophotometer (Infinite M200 Pro; Tecan Group Ltd., Mannedorf, Switzerland). The total polyphenol content was expressed as gallic acid equivalents (GAE) based on a gallic acid standard calibration curve.

To determine the total flavonoid content, 1 mL of the appropriately diluted sample extract was mixed with 1 mL of 2% aluminum chloride solution in methanol and incubated at room temperature for 15 min. Absorbance was measured at 430 nm and the total flavonoid content was expressed as quercetin equivalents (QE) using a quercetin standard calibration curve.

2.9. Measurement of antioxidant activity

To evaluate the antioxidant activity, 5 g of powdered cookies were mixed with 20 mL of 95% ethanol using a

https://doi.org/10.11002/fsp.2025.32.5.823

vortex mixer for thorough homogenization. The mixture was centrifuged at 13,500 ×*g* for 10 min, and the resulting supernatant was used for the antioxidant assays.

The DPPH radical scavenging activity was determined according to the method described by Cheung et al. (2003). Briefly, 100 μL of cookie extract was mixed with 100 μL of 0.2 mM DPPH solution and incubated at 37°C for 30 min. After incubation, absorbance was measured at 515 nm using a spectrophotometer. Scavenging activity was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = (1 - A_{sample} / A_{control}) \times 100$$

where A_{sample} is the absorbance of the sample with the extract and $A_{control}$ is the absorbance of the control without the extract.

ABTS radical scavenging activity was measured as described by Re et al. (1999). $ABTS^{+}$ radicals were generated by mixing 7.0 mM ABTS solution with 2.45 mM potassium persulfate and incubating the mixture in the dark for 24 h. Prior to use, the $ABTS^{+}$ solution was diluted with ethanol to obtain an absorbance of 0.17±0.03 at 735 nm. Then, 100 μL of the diluted $ABTS^{+}$ solution was mixed with 100 μL of cookie extract and incubated at 37°C for 30 min. Absorbance was measured at 732 nm and scavenging activity was calculated using the following equation:

$$\text{ABTS radical scavenging activity (\%)} = (1 - A_{sample} / A_{control}) \times 100$$

The reducing powers of the samples were measured as described by Oyaizu (1986). Briefly, cookie extract (0.5 mL) was mixed sequentially with 20 mM phosphate buffer (pH 6.6) and 0.5 mL 1% potassium ferricyanide solution (0.5 mL). The mixture was incubated at 50°C for 20 min. After incubation, 1 mL of 10% trichloroacetic acid was added and mixed well, followed by centrifugation at 9,000 rpm for 15 min. One milliliter of the supernatant was combined with 1 mL each of distilled water and 1 mL of 0.1% ferric chloride solution, mixed, and allowed to react at room temperature. Absorbance was measured at 720 nm, with higher absorbance values indicating greater reducing power.

2.10. Statistical analysis

All experiments were performed in triplicate under identical conditions, and the results are expressed as mean±standard deviation. Statistical analyses were conducted using R-Studio software (Version 3.5.1; Boston, MA, USA). Differences among treatment groups were evaluated using one-way analysis of variance (ANOVA) and Student's t-test. When significant differences were detected, Duncan's multiple range test was performed for post-hoc comparisons. Statistical significance was set at $p<0.05$.

3. Results and discussion

3.1. Spread factor, leavening rate, and baking loss rate of cookies

The spread factor, leavening rate, and baking loss rate of cookies prepared with varying amounts of BSG powder are shown in Table 2. These parameters tended to decrease proportionally with increasing BSG powder content compared with that in the control group. The highest spread factor was observed in the control cookies (4.30), whereas cookies

Table 2. Spread factor, leavening rate, loss rate and hardness of cookies fortified with brewer's spent grain powder

Sample	Measurement		
	Spread factor	Leavening rate (%)	Loss rate (%)
0	4.30±0.10[1)a2)]	100.00±0.00[a]	18.97±0.23[a]
10	4.26±0.09[a]	85.45±4.98[b]	15.83±1.03[b]
20	4.22±0.11[a]	83.64±4.07[b]	15.60±0.80[b]
30	4.02±0.04[b]	69.09±4.98[c]	12.94±1.07[c]
40	3.98±0.20[b]	67.27±4.98[c]	12.75±0.76[c]

[1)]All values are mean±SD (n=3).
[2)]Means with different superscript letters ([a-c]) in the same column are significantly different ($p<0.05$) by Duncan's multiple range test.

containing 10% and 30% BSG powder exhibited reduced spread factors of 4.26 and 4.02, respectively. The lowest spread factor (3.98) was observed in cookies containing 40% BSG powder.

Using the leavening rate of the control cookies (without BSG powder) as 100%, the leavening rates of cookies supplemented with 10-40% BSG powder decreased progressively from 85.45% to 62.27%. Baking loss rate was the highest in the control group (18.97%), whereas cookies containing 10% and 20% BSG showed reduced baking loss rates of 15.83% and 15.60%, respectively, with no statistically significant difference between these two groups. Cookies containing 30% and 40% BSG exhibited the lowest baking loss rates of 12.94% and 12.75%, respectively.

Cookies prepared with 10-40% BSG powder demonstrated a gradual decrease in the spread factor, leavening rate, and baking loss rate as the substitution level increased. This trend was attributed to the fiber and protein content of BSG interfering with gluten formation and water absorption, thereby inhibiting dough expansion and rising (Kteioudaki et al., 2012; Waters et al., 2012). The decrease in the spread factor is believed to result from the dietary fiber in BSG absorbing water within the dough, increasing its viscoelasticity and limiting thermal spread during baking. Similar observations have been reported when part of the flour was replaced with fiber-rich ingredients, such as rice bran or barley grass (Lim, 2022; Mishra, 2017).

The reduction in the leavening rate can be explained by the increased density of proteins and dietary fibers in the dough upon heating, which hinders gas formation and expansion (Kteioudaki et al., 2012; Mishra, 2017). The baking loss rate, which reflects moisture evaporation during baking, was reduced in the BSG-supplemented cookies, likely because BSG strongly binds moisture within the dough, suppressing water loss during baking (Nam et al., 2023). Consistent with these results, previous studies have reported decreased baking loss rates in cookies enriched with fiber-rich ingredients, such as green tea powder (Hwang and Park, 2021), perilla seed meal (Oh et al., 2022), and onion peel powder (Yeom and Hwang, 2020).

3.2. Proximate composition analysis

The proximate compositions of BSG, flour, and cookies prepared with varying amounts of BSG powder are summarized in Table 3. The moisture content of BSG was 0.20%, which was significantly lower than that of the flour (8.57%). Conversely, the ash content of the BSG was 2.04%, which was markedly higher than the 0.18% content observed in the flour. The crude protein and fat contents of BSG were 0.56% and 1.65%, respectively.

The control group (without BSG) exhibited the highest moisture content of 3.23%, whereas the moisture content decreased gradually from 3.16% to 2.21% as the BSG substitution level increased. This reduction was attributed to the lower moisture content of the BSG powder than that of the flour, which reduced the overall water retention capacity of the dough. The crude protein content increased from

Table 3. Proximate analysis of Brewer's Spent-grain powder, wheat flour and cookies fortified with brewer's spent grain powder

Sample	Moisture	Crude protein	Crude fat	Ash
Brewer's spent-grain powder	0.20±0.11[1)]	0.56±0.00	1.65±2.12	2.04±0.10
Wheat flour	8.57±0.98[*2)]	0.49±0.02[*]	2.86±2.53[ns3)]	0.18±0.06[*]
Cookies made by brewer's spent-grain powder (%)				
0	3.23±0.22[a4)]	0.19±0.00[d]	19.77±0.82[ns]	0.47±0.04[b]
10	3.16±0.43[a]	0.21±0.01[c]	20.37±0.34	0.53±0.10[b]
20	2.70±0.12[ab]	0.22±0.00[bc]	20.78±1.07	0.79±0.03[a]
30	2.62±0.18[ab]	0.24±0.01[b]	19.73±1.59	0.92±0.06[a]
40	2.21±0.14[b]	0.26±0.00[a]	21.33±0.14	0.91±0.06[a]

[1)]All values are mean±SD (n=3).
[2)]Significant differences at $p<0.05$ by Student's t-test.
[3)ns], not significant.
[4)]Means with different superscript letters ([a-d]) in the same column are significantly different ($p<0.05$) by Duncan's multiple range test.

https://doi.org/10.11002/fsp.2025.32.5.823

0.19% to 0.26% as the BSG content increased, reflecting the higher protein content of BSG than that of flour.

The crude fat content ranged from 19.77% to 21.33% across all cookies, with no statistically significant differences observed between the control and experimental groups. This lack of variation is likely due to the consistent use of lipid sources, such as eggs and butter in all formulations, which, alongside the flour and BSG content, collectively influenced the fat content of the final products. Given the uniform amount of fat across all samples, the total fat content remained relatively constant. The ash content of the cookies increased significantly from 0.47% in the control to 0.53% and 0.91% with increasing BSG addition. This is interpreted as a consequence of the relatively higher ash content in BSG than in the flour.

3.3. Soluble solids, pH, acidity, and hardness

The soluble solids, pH, acidity, and hardness of cookies prepared with varying levels of BSG powder are presented in Table 4. The soluble solid contents of the BSG and flour used in this study were 5.25 and 1.45 °Brix, respectively, indicating that BSG had a higher soluble solid content than flour. The control cookies without BSG had a soluble solid content of 4.70 °Brix, which increased proportionally with BSG addition from 4.90 to 5.75 °Brix as the substitution level increased from 10% to 40%.

The pH values of the extracts from BSG and flour were 5.91 and 6.13, respectively, with BSG exhibiting a lower pH than flour. The pH of the cookies ranged from 7.30 in the control to 6.84-6.58 as the BSG content increased from 10% to 40%, likely reflecting the lower pH of the BSG powder than that of the flour. The acidity values of the BSG and flour were 0.20% and 0.06%, respectively, with BSG exhibiting the highest acidity. The acidity of the cookies was the lowest at 0.01% in the control group and increased from 0.03% at 10% BSG addition to 0.09% at 40% BSG addition.

The increase in soluble solid content can be attributed to the transfer of residual monosaccharides, water-soluble fibers, and protein hydrolysates present in BSG into the cookie matrix during heat treatment, resulting in a higher total soluble solid concentration in the dough (Kteioudaki et al., 2012; MinAlexander et al., 2023). BSG contains various soluble solids, such as glucans, amino acids, peptides, and sugars (MinAlexander et al., 2023), and metabolites from hops, yeast, and malt used in brewing are concentrated in BSG, resulting in a higher organic acid content and lower pH (Pires et al., 2014). Consequently, higher BSG content increased cookie acidity and decreased pH. Similar findings were reported by Waters et al. (2012), who observed significant pH reduction and increased acidity in baked goods fortified with BSG powder. These results suggest that BSG addition is a key factor in increasing the soluble solid concentration and acidity of cookies, potentially affecting the flavor, microbial stability, and shelf life.

Hardness measurements showed an increasing trend with higher BSG addition. The control cookies exhibited the

Table 4. Sugar content, pH, total acidity and hardness of brewer's spent-grain powder, wheat flour and cookies fortified with brewer's spent grain powder

Sample	Sugar content (°Brix)	pH	Total acidity (%)	Hardness (g)
Brewer's spent-grain powder	5.25±0.07[1)]	5.91±0.00	0.20±0.03	ND[3)]
Wheat flour	1.45±0.07[*2)]	6.13±0.01[*]	0.06±0.00[*]	ND
Cookies made by brewer's spent-grain powder (%)				
0	4.70±0.00[e4)]	7.30±0.00[e]	0.01±0.00[e]	11447.33±89.00[c]
10	4.90±0.00[d]	6.84±0.00[d]	0.03±0.00[d]	11811.00±79.77[b]
20	5.20±0.00[c]	6.66±0.00[c]	0.05±0.00[c]	11973.33±28.45[a]
30	5.30±0.00[b]	6.62±0.01[b]	0.06±0.00[b]	11987.33±62.07[a]
40	5.75±0.07[a]	6.58±0.00[a]	0.09±0.00[a]	12068.00±42.04[a]

1)All values are mean±SD (n=3).
2)Significant differences at $p<0.05$ by Student's t-test.
3)ND, not determined.
4)Means with different superscript letters (a-e) in the same column are significantly different ($p<0.05$) by Duncan's multiple range test.

lowest hardness at 11,447.33 g, which increased to 11,811.00 g with the addition of 10% BSG. Cookies with 20-40% BSG showed hardness values ranging from 11,973.33 to 12,068.00 g, which were significantly higher than those of the control and 10% BSG groups, although no statistically significant differences were found among the 20-40% BSG groups.

BSG mainly consists of insoluble dietary fibers and residual proteins, which rapidly absorb moisture and reduce dough viscoelasticity, resulting in a dense cookie texture (Waters et al., 2012). This leads to suppressed bubble formation during baking and accelerated moisture loss during heat transfer, ultimately increasing hardness (Gul et al., 2017; Saberian et al., 2024). Nicolai et al. (2025) reported that the addition of BSG to bakery products significantly increases the hardness and brittleness of cookies. Jiang et al. (2019) similarly found that fiber-rich alternative ingredients weakened the gluten network and reduced water activity in dough, resulting in a denser structure and higher hardness, thus validating the findings of this study.

Although a formal sensory evaluation was not performed in this study, the observed alterations in texture and acidity with higher levels of BSG substitution could potentially influence consumer acceptability. Previous studies have suggested that firmer texture in bakery products may be associated with reduced consumer preference, whereas moderate increases in acidity could enhance flavor perception. Therefore, while the present findings highlight the technological potential of BSG incorporation, future research incorporating sensory panels will be essential to fully assess consumer perception and market feasibility.

3.4. Color and browning index

The color characteristics of BSG, wheat flour, and cookies formulated with different levels of BSG substitution are presented in Fig. 1 and summarized in Table 5. The lightness values of BSG and flour were 64.60 and 94.40, respectively, indicating that BSG exhibited a darker color than flour. The control sample without BSG had the highest lightness value of 74.50. As the BSG content increased from 10% to 40%, lightness gradually decreased from 57.54 to 43.72.

The redness values of BSG powder and flour were 2.31 and -2.03, respectively, with BSG powder exhibiting higher redness than flour. The control cookies had the lowest redness value at -2.75, and redness values increased proportionally with BSG content from 1.82 to 4.07. The yellowness values were 14.15 for BSG powder and 9.38 for flour, indicating higher yellowness in BSG than in flour. The yellowness of the control cookies were the highest at 23.31 but decreased to 17.71 and 16.87 with 10% and 20% BSG addition, respectively, and further decreased to 15.38 and 15.13 with 30% and 40% BSG addition, respectively.

The observed decrease in lightness and yellowness, along with the increase in redness with increasing BSG content, can be attributed to the intrinsic brown to reddish pigments in BSG, as well as the polyphenols, proteins, and fibers present in BSG, which promote Maillard and caramelization reactions during heat treatment (Hwang and Park, 2021; Starowicz and Zielinski, 2019). The decrease in lightness indicated a darker surface color of the cookies, resulting from the high pigment concentration in BSG and browning caused by interactions between amino acids and sugars. The increase in redness was associated with the formation of brown pigments, which is consistent with reports on bakery products fortified with similar cereal byproducts or plant-based high-fiber ingredients (Ngo et al., 2024). The decrease in yellowness likely reflects the transition from a bright yellowish color typical of

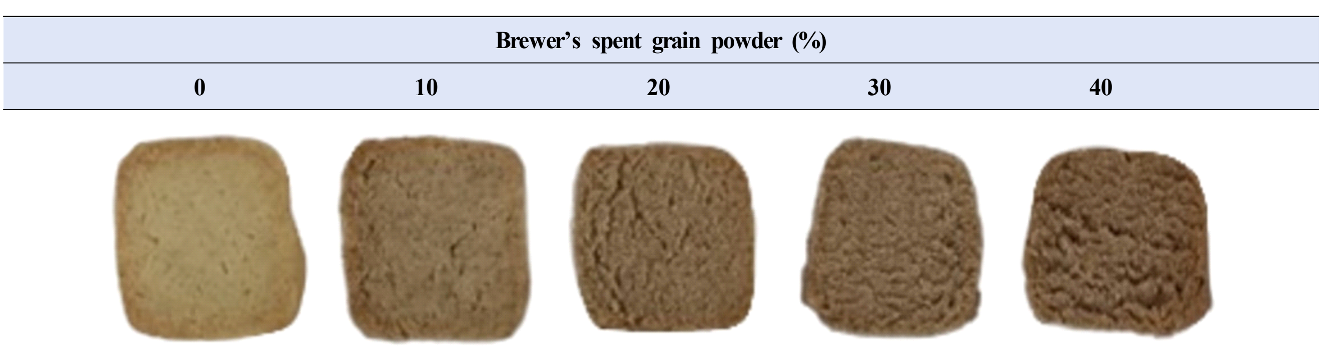

Fig. 1. **Cookies fortified with brewer's spent grain powder.**

https://doi.org/10.11002/fsp.2025.32.5.823

Table 5. Changes in Hunter's color value and browning index of brewer's spent-grain powder, wheat flour and cookies fortified with brewer's spent grain powder

Sample	L	a	b	BI[1)]
Brewer's spent-grain powder	64.60±0.14[2)]	2.31±0.08	14.15±0.16	ND[4)]
Wheat flour	94.40±0.16[*3)]	-2.03±0.00[*]	9.38±0.03[*]	ND
Cookies made by brewer's spent-grain powder (%)				
0	74.50±0.05[a5)]	-2.75±0.16[e]	23.31±0.18[a]	199.34±0.65[e]
10	57.54±0.19[b]	1.82±0.06[d]	17.71±0.11[b]	216.02±0.20[d]
20	51.33±0.17[c]	3.38±0.14[c]	16.87±0.11[c]	222.57±0.45[c]
30	45.78±0.16[d]	3.80±0.18[b]	15.38±0.14[d]	225.51±0.58[b]
40	43.72±0.14[e]	4.07±0.09[a]	15.13±0.13[e]	226.89±0.21[a]

1)BI, browning index.
2)All values are mean±SD (n=3).
3)Significant differences at $p<0.05$ by Student's t-test.
4)ND, not determined.
5)Means with different superscript letters ([a-e]) in the same column are significantly different ($p<0.05$) by Duncan's multiple range test.

flour-based cookies to a darker and redder hue owing to the addition of BSG.

The BI calculated from the color values showed the lowest value of 199.34 in the control group, increasing proportionally with the BSG content. Cookies containing 10-40% BSG had BI values ranging from 216.02 to 226.89. Browning index serves as an indicator of non-enzymatic browning, primarily through the Maillard and caramelization reactions. These browning reactions are not merely visual changes but are closely linked to antioxidant properties (Starowicz and Zielinski, 2019). Previous studies have shown that Maillard reaction products possess DPPH and ABTS radical scavenging activities and are positively correlated with antioxidant indices (Hwang and Park, 2021). For example, Ajila et al. (2008) reported that cookies supplemented with mango peel powder showed increased BI, elevated total phenolic content, and DPPH radical scavenging activity. Similarly, Karpinska-Tymoszczyk et al. (2024) observed enhanced antioxidant activity in cookies with high browning levels fortified with sea buckthornberries.

These findings suggest that active Maillard reactions generate abundant antioxidant intermediates or final products within the samples. However, an increase in the browning index does not necessarily guarantee improved functionality, as excessive browning can degrade the quality and induce the formation of harmful substances, such as acrylamide. Therefore, it is essential to control the degree of browning within an appropriate range (Hee et al., 2024; Hwang and Park, 2021).

3.5. Total polyphenol and total flavonoid contents

The total polyphenol and total flavonoid contents of cookies manufactured with varying levels of BSG are presented in Table 6. The control cookies without BSG addition had a total polyphenol content of 35.05 μg of gallic acid equivalent (GAE)/g. As the BSG content increased, the total polyphenol content of the cookies also increased. Specifically, cookies with 10% and 20% BSG additions exhibited total polyphenol contents of 63.78 μg and 84.39 μg GAE/g, representing 1.82- and 2.41-fold increases compared to the control. Cookies containing 30% and 40% BSG showed even higher polyphenol contents at 117.18 μg and 149.33 μg GAE/g, corresponding to 3.34- to 4.26-fold increases relative to the control group, respectively.

Ethanol extracts of the BSG and flour used in this study contained total polyphenol contents of 177.00 μg and 44.58 μg GAE/g, respectively, indicating that BSG contained approximately 3.97 times higher polyphenol content than flour. This explains the proportional increase in total polyphenol content in cookies with increasing BSG addition.

Regarding total flavonoid content, the control cookies without BSG had 4.53 μg quercetin equivalent (QE)/g of sample. The total flavonoid content increased with BSG addition, reaching 7.68, 13.71, 15.07, and 18.35 μg QE/g for

Table 6. **Total polyphenol and total flavonoid contents of brewer's spent-grain powder, wheat flour and cookies fortified with brewer's spent grain powder**

Sample	Total polyphenol (μg GAE[1)]/g)	Total flavonoid (μg QE[2)]/g)
Brewer's spent-grain powder	177.00±1.46[3)]	37.57±1.28
Wheat flour	44.58±0.75[*4)]	16.74±0.74[*]
Cookies made by brewer's spent-grain powder (%)		
0	35.05±1.63[e5)]	4.53±0.33[e]
10	63.78±0.59[d]	7.68±0.26[d]
20	84.39±0.92[c]	13.71±1.07[c]
30	117.18±0.85[b]	15.07±0.53[b]
40	149.33±0.84[a]	18.35±0.48[a]

1)GAE, gallic acid equivalent.
2)QE, quercetin equivalent.
3)All values are mean±SD (n=3).
4)Significant differences at p<0.05 by Student's t-test.
5)Means with different superscript letters ([a-e]) in the same column are significantly different (p<0.05) by Duncan's multiple range test.

cookies with 10%, 20%, 30%, and 40% BSG, respectively. These values represent a 1.70- to 4.05-fold increase compared with the control. The ethanol extracts of BSG and flour contained 37.57 μg and 16.74 μg QE/g, respectively, indicating that the BSG powder contained approximately 2.24 times higher total flavonoid content than flour. This accounts for the increased flavonoid content of cookies in proportion to the addition of BSG.

BSG retains a significant amount of the bioactive compounds generated during beer brewing, in which hops, malt, and yeast are subjected to high-temperature extraction and fermentation. It is particularly rich in phenolic compounds, such as ferulic acid, *p*-coumaric acid, catechin, and quercetin, which contribute to enhanced antioxidant activity and functional properties in processed foods proportional to BSG content (Saberian et al., 2024). Merten et al. (2022) reported that replacing flour with 10% to 100% BSG in bread production significantly increased total polyphenol and flavonoid contents, as well as antioxidant activities, consistent with the present findings.

3.6. Antioxidant activities

The antioxidant activities of BSG, flour, and cookies manufactured with varying BSG contents were evaluated by measuring the DPPH and ABTS radical scavenging activities and reducing power, as shown in Table 7. The DPPH radical scavenging activities of the ethanol extracts of BSG and flour were 42.93% and 41.63%, respectively, indicating no significant differences between them. The DPPH radical scavenging activity of cookies without BSG (control) was 31.93%, whereas cookies with 10-40% BSG showed increased activities ranging from 36.86% to 40.49%, approximately 1.15-1.27-fold higher than that of the control. These values were also higher than those of ascorbic acid (20 μg/mL), which exhibited 33.55% scavenging activity.

The ABTS radical scavenging activity of the control cookies without BSG was the lowest (34.83%). The ABTS radical scavenging activity increased proportionally with BSG content, reaching 49.11%, 60.22%, 72.08%, and 77.18% for cookies containing 10%, 20%, 30%, and 40% BSG, respectively. This represented a 1.41-2.22-fold increase compared to the control and exceeded the 44.83% activity of ascorbic acid (20 μg/mL). The ABTS radical scavenging activities of the ethanol extracts from flour and BSG were 4.36% and 68.92%, respectively, demonstrating that BSG exhibited approximately 15.81 times higher ABTS scavenging activity than flour. This explains the proportional increase in the ABTS radical scavenging activity of cookies with increasing BSG addition.

The reducing power showed a trend similar to that of the DPPH and ABTS radical scavenging activities. The control cookies without BSG had the lowest reducing power at 0.17, which increased to 0.33-0.74 with 10-40% BSG addition. The reducing powers of ethanol extracts from BSG and flour were 0.57 and 0.14, respectively, indicating that the higher reducing power of BSG contributed to the increased reducing power in cookies as BSG content increased.

In this study, although the DPPH radical scavenging activity increased compared to that of the control upon BSG addition, no significant difference was observed among the varying BSG levels. In contrast, the ABTS radical scavenging activity and reducing power significantly increased with BSG addition. This difference might be because the DPPH assay primarily detects lipophilic antioxidants, whereas the ABTS assay is a more sensitive method that evaluates both hydrophilic and lipophilic antioxidant components (Floegel et al., 2011; Re et al., 1999). BSG contains abundant water-soluble antioxidant components, such as polyphenols, amino acids, and dietary fibers, which react sensitively with ABTS radicals and reducing power assays, but may show lower reactivity in the DPPH assay (Prior et al., 2005). Additionally,

https://doi.org/10.11002/fsp.2025.32.5.823

Table 7. Antioxidant activities of brewer's spent-grain powder, wheat flour and cookies fortified with brewer's spent grain powder

Sample	DPPH radical scavenging (%)	ABTS radical scavenging (%)	Reducing power
Brewer's spent-grain powder	42.93±0.51[1)]	76.87±0.68	0.57±0.04
Wheat flour	41.63±0.38[*2)]	35.50±1.72[*]	0.14±0.00[*]
Cookies made by brewer's spent-grain powder (%)			
0	31.93±1.26[c3)]	34.83±0.91[e]	0.17±0.00[e]
10	36.86±0.97[b]	49.11±0.57[d]	0.33±0.01[d]
20	39.35±1.20[a]	60.22±0.80[c]	0.57±0.02[c]
30	39.87±1.63[a]	72.08±0.75[b]	0.66±0.01[b]
40	40.49±1.15[a]	77.18±0.42[a]	0.74±0.04[a]
Ascorbic acid 20 μg/mL	33.55±1.15	44.83±0.82	0.47±0.00

1)All values are mean±SD (n=3).
2)Significant differences at $p<0.05$ by Student's t-test.
3)Means with different superscript letters (a-c) in the same column are significantly different ($p<0.05$) by Duncan's multiple range test.

the increase in reducing power is related to the ability of phenolic compounds and protein hydrolysates in BSG to act as reductants by converting ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), reflecting the increased total antioxidant content proportional to BSG addition (Oyaizu, 1986; Prior et al., 2005).

These findings highlight that the choice of the antioxidant assay can influence the observed antioxidant properties of the samples. Therefore, for the comprehensive quantitative evaluation of BSG powder as a functional ingredient, multiple complementary antioxidant assays should be employed.

4. Conclusions

This study aimed to develop functional cookies utilizing BSG and evaluate the physicochemical quality characteristics and antioxidant activities of cookies with varying BSG powder content (10-40%). Physical property analysis revealed that increasing the BSG content significantly decreased cookies' spread factor, leavening rate, and weight loss, which can be attributed to the dietary fiber and protein in BSG interfering with gluten formation and moisture absorption, thereby reducing dough viscosity and expansion. Cookie hardness increased proportionally with BSG addition. As the BSG content increased, cookie lightness and yellowness decreased, whereas redness and browning index increased, indicating more pronounced browning. Proximate analysis showed that cookies with BSG had significantly higher ash and crude protein contents and lower moisture content than the control. With increasing BSG content, soluble solids increased, pH decreased, and acidity increased. The total polyphenol and flavonoid contents increased 4.26- and 4.05-fold, respectively, in proportion to BSG addition. Evaluation of antioxidant activity revealed a slight increase in DPPH radical scavenging activity compared to the control, whereas ABTS radical scavenging activity and reducing power markedly increased with BSG addition. These trends are interpreted to result from the water-soluble antioxidant components in BSG that respond sensitively to antioxidant assays, with observed variations depending on the analytical method used. BSG powder was confirmed to affect the structural and quality attributes of cookies while effectively enhancing the content of functional antioxidant compounds. This study suggests practical applications for valorizing food byproducts and indicates that BSG is a promising natural ingredient for the development of functional bakery products.

Funding

None.

Acknowledgements

None.

Conflict of interests

The authors declare no potential conflicts of interest.

Author contributions

Conceptualization: Hwang ES. Methodology: Hwang ES, Cheung J. Formal analysis: Hwang ES, Cheung J. Validation: Hwang ES. Writing - original draft: Hwang ES. Writing - review & editing: Hwang ES.

Ethics approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

ORCID

Jisung Cheung (First author)
https://orcid.org/0009-0008-1414-0123
Eun-Sun Hwang (Corresponding author)
https://orcid.org/0000-0001-6920-3330

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