**Biovalorisation of brewer's spent grain (BSG) and sensory
evaluation of BSG bread**

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Abstract

Brewer's spent grain (BSG) is the largest by-product of beer production, generating over an approximate 38 billion kg annually. While the majority of BSG gets repurposed as animal feed, its usage unfortunately remains very limited. Due to the impressive nutritional profile of BSG, many studies have investigated its incorporation in food products. However, its substitution at high levels tend to bring about undesirable sensory changes. This paper looks at solid-state fermentation as a tool to enhance the nutritional profile of BSG. The consumer acceptance of fermented BSG-fortified bread was investigated, to understand the market value for fermented BSG food products compared to its unfermented counterpart. Of the 8 *koji* starters studied, M-1 (*Aspergillus oryzae*) brought about the greatest nutritional profile enhancement in terms of total phenolic content and crude protein content, with an optimal fermentation time of 4 days. No change in total dietary fibre content was observed after fermentation. From the sensory evaluation, fermented BSG-fortified bread had the best nutritional profile while having the poorest consumer acceptance. Despite the fact, this study highlights that fermentation may yet be an important tool in bridging the gap of BSG incorporation in food.

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Chapter 1 Introduction

1.1 Background

Having an estimated production of 1.91 billion hectolitres in 2019 alone,^[1] beer is one of the most popular beverages consumed worldwide, just after water and tea. Beer, however, generates large quantities of by-products in the form of brewer's spent grain (BSG), spent hops and surplus yeast. BSG is the leftover, insoluble portions of the barley grain (*Hordeum vulgare*), accounting for an approximate 85% of all waste generated during the brewing process.^[2] For every hectolitre of beer produced, an estimated 20 kg of BSG is generated,^[3] amounting to over 38 billion kg of BSG waste produced in 2019. BSG is relatively limited in terms of application, with majority of it used as low-value animal fodder.^[4] Many breweries have chosen to either compost or directly apply it to soil to improve soil quality. Some have even chosen to convert their waste to energy through processes such as anaerobic digestion and incineration.^[4-5] However, an estimated 20% of BSG is still sent to landfill,^[6] as many small breweries do not have the means to valorise their waste. Given that landfills are a significant source of anthropogenic methane,^[7] the BSG sent to landfill should be minimised, in order to curb its contribution to environmental degradation.

1.2 The brewing process

BSG is an inherently inconsistent by-product.^[8] Its composition varies significantly between breweries and the types of beer produced, due to a variety of factors. To understand them, we will first have to look at the brewing process. During the production of beer, barley first goes through a germination process called 'malting'. Malting hydrates the barley grains and promotes the synthesis and activation of enzymes such as amylases, proteases and β -glucanases.^[3] Following this, malted barley is dried, milled and undergoes a process known as 'mashing'. During mashing, malted barley is mixed with large volumes of water at high temperatures. The activated enzymes then break down the starches, proteins and arabinoxylans, producing both fermentable and non-fermentable sugars.^[3] After mashing, a sugary liquid known as

'wort' is filtered out to be used as the fermentation medium for brewing. The leftover solid residue after filtration is BSG. As a result, the differences in malting and mashing conditions, barley variety, and harvest time, all contribute to the differences in the composition of BSG.^[8]

When fresh, BSG is considered safe for consumption due to the high temperatures from mashing. However, it is susceptible to rapid microbial degradation due to its high moisture content.^[6] To increase the shelf life of BSG, it needs to be either frozen, dried or acidified, all of which presents its own set of benefits and limitations.^[3] This is thus one of the reasons why BSG is under-utilised and limited in terms of application.

1.3 Nutritional profile of BSG

Despite the variance in the composition of BSG, it is undoubtedly an excellent source of cellulose, hemicellulose and lignin. When dried, BSG consists of approximately 70% fibre.^[3] As a result, incorporating BSG into food products typically improves their dietary fibre content significantly.^[9] Higher dietary fibre intake has shown to improve stool production and bulk, decrease low-density lipoprotein (LDL) cholesterol levels and/or blood total, and modulate blood glucose levels.^[10] BSG contains an estimated 20% protein when dried and is a good source of essential and nonessential amino acids.^[3] Additionally, it is also a rich source of phenolic compounds,^[11] which are naturally occurring aromatic compounds associated with antioxidant capabilities. Hydroxycinnamic acids (HCAs) are the main source of phenolic compounds in BSG, which constitute *p*-coumaric, sinapic, caffeic and ferulic acids.^[2] Studies on HCAs have shown numerous benefits for human health, such as antitumor, anti-inflammatory, anti-platelet aggregation, antidiabetic, and antimicrobial properties.^[12]

1.4 Solid-state fermentation

Since BSG is an excellent source of many desirable compounds and nutrients, previous studies have explored biovalorisation through solid-state fermentation (SSF) to either extract these compounds or enhance its nutritional value.^[13] SSF is fermentation carried out in the lack of free water. The fermentation medium, however,

should still contain enough moisture for microorganisms to grow.^[14] SSF is an ancient fermentation technique traditionally used in Asian countries to produce fermented food and beverages such as soy sauce, saké, tempeh, and Shaoxing wine.^[15] In Japan, fermentation starters are known as *koji* starters, which consist of steamed grains inoculated with a filamentous fungus known as *Aspergillus oryzae*.^[15] Less common species such as *A. luchuensis*, *A. awamori* and *A. sojae* are also used as *koji* starters. Fermentation is generally used to preserve food, improve its quality and safety, develop unique textures and flavours, and improve its nutritional profile.^[16] Studies on the SSF of BSG have reported increases in total phenolic content (TPC), anti-oxidant activity (DPPH),^[17] amino acids, and vitamins, while reducing fats and carbohydrates.^[18]

The main alternative of SSF is submerged fermentation (SmF), whereby fermentation is carried out in a liquid medium with a large amount of free water.^[19] SSF, however, more accurately mimics the natural environment of fungi and moulds such as *A. oryzae*, making it the preferred fermentation method for their growth.^[20] There are many benefits from using SSF over SmF for fermentation using fungi and moulds, including higher concentration of desired products, lower need for sterility due to lower moisture content, sufficient supply of oxygen, and higher sustainability due to lower organic wastewater production.^[21]

1.5 Sensory evaluation

Given the impressive nutritional profile of BSG and push for more sustainable circular food systems, previous studies have looked at incorporating it in food. These studies have explored the incorporation of BSG into baked products such as muffins,^[22] cookies, biscuits, granola bars,^[23] pasta,^[9a] and bread.^[9b] The first of its kind, Waters, *et al.* (2012) conducted a sensory evaluation of fermented BSG sourdough bread.^[24] These studies have demonstrated improvements in the nutritional profile of their products, in terms of increased amino acids, dietary fibre, protein levels, TPC, and decreased calorie content.^[9, 22, 25] Despite the successful substitution of BSG at lower concentrations, higher substitution concentrations result in an increase in undesirable

sensory properties. These include increased acidulous aroma and flavour, increased colour intensity, along with decreased sweetness and consumer acceptability.^[24-26]

1.6 Objectives and hypotheses

In order to circularise the beer brewing process, BSG has to find its way back into food. However, the incorporation of BSG into food products has shown to decrease consumer acceptability, due to its increasing undesirable sensory properties. As a result, this paper aims to improve the nutritional profile of BSG through fermentation, as well as improve the consumer acceptability of BSG-fortified food through fermentation.

We expect an improvement in the nutritional profile of BSG after SSF using Japanese food microbes, across the parameters of TPC, crude protein and dietary fibre. It is hypothesised that the consumer acceptance of fermented BSG in baked products would be similar compared to its unfermented or control counterpart. Lastly, the nutritional profile of the fermented BSG baked products are expected to be more desirable compared to its unfermented counterpart.

Chapter 2 Materials and Methods

2.1 Materials

All fresh BSG was kindly provided by local brewery Brewerkz Brewing Co. (Singapore) Pte Ltd. and stored in airtight Ziploc bags until use. The *koji* starters EM-2, M-1, SP-01, SP-05, NJK110 and Fuji and were purchased from Nihon Jyozo Kogyo (Tokyo, Japan). The *koji* starter Shōchū Black Koji was purchased from Akita Konno Shoten, and the *koji* starter *Aspergillus oryzae* Hashimoto was purchased from Higuchi Moyashi. EM-2, M-1, SP-05, NJK110, Fuji and Hashimoto were composed of the spores of *A. oryzae*. SP-01 was composed of the spores of *A. oryzae* and *A. sojae*. Shōchū Black Koji was composed of the spores of *A. luchuensis*.

Folin & Ciocalteu's phenol (FC) reagent, gallic acid, tris(hydroxymethyl)aminomethane (TRIS), 2(*N*-morpholino) ethanesulfonic acid (MES), methyl red and bromocresol green were purchased from Sigma-Aldrich (St. Louis, MO, USA). Boric acid was purchased from Merck (Lebanon, NJ, USA). Sodium carbonate anhydrous (Na_2CO_3) was purchased from QREC (Rawang, Selangor, Malaysia). Concentrated sulfuric acid was purchased from Kanto Chemical (Marunouchi, Tokyo, Japan). Acetone and ethanol (technical grade) were purchased from Aik Moh (Singapore). HPLC grade methanol and 37% hydrochloric acid were purchased from VWR International (Rue Carnot, France). Kjeltabs (3.5 g K_2SO_4 , 0.4 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) were purchased from FOSS (Hillerød, Denmark). Sodium hydroxide (NaOH) pellets were purchased from VWR International (Leuven, Belgium). Purified Milli-Q water of 18 mΩ-cm resistivity was used for the preparation for all buffers and solutions.

2.2 Pre-treatment of BSG

Pre-treatment of BSG was carried out before the SSF (see 2.3) and bread making procedure (see 2.7). Frozen BSG was thawed, then dehydrated overnight in a 60 °C oven (France Etuves XU058, Chelles, France) until completely dried. The samples were then collected and grinded (Gewürz & Kaffee Mühle, Rommelsbacher,

Germany), followed by sieving through a 30-mesh sieve ($\leq 595 \mu\text{m}$ particle size). The samples were then autoclaved (Sanyo Labo Autoclave, Osaka, Japan) twice at 121°C for 15 min, bringing moisture content to $4.37 \pm 1.15\%$ wet basis (w.b.).

2.3 Solid-state fermentation of BSG

To prepare the controls, 8 g of the pre-treated BSG was rehydrated with 12 g of sterile deionised water. To prepare the inoculated samples, 8 g of the pre-treated BSG was rehydrated with 11.6 g of sterile deionised water, followed by inoculation using 0.4 mL of a pre-prepared spore suspension. Both the controls and inoculated samples would have 60% w.b. moisture content. All spore suspensions were freshly prepared on the day of inoculation with a concentration of 10^6 spores per gram of dry matter, and were vortexed (Scientific Industries, New York, USA) for a minimum of 10 min to ensure good suspension. After inoculation, the samples were placed in the dark to ferment at 22.5°C and 52% humidity. When fermentation was complete, the samples were dehydrated overnight in a food dehydrator (Excalibur Food Dehydrators, CA, USA) at 67°C . When completely dried, the samples were ground using a grinder for 30 s and stored at 4°C until needed for analysis.

2.4 Total phenolic content analysis

The total phenolic content (TPC) was determined using the Folin-Ciocalteu (FC) method^[27], adapted for microplates. 200 mg of the samples were vortexed well with 1 mL of 50% methanol (v/v), followed by centrifugation (15,000 rpm, 10 min). 10 μL of the supernatants from each sample were extracted and diluted 50 times using deionised water. 100 μL of the diluted supernatant, 1 mL of a 10-times diluted FC solution, followed by 800 μL of 1M Na_2CO_3 solution were added respectively into individual 4 mL glass vials. The vials were then incubated for 15 min at 45°C (Ecotherm, Torrey Pines Scientific Inc., CA, USA). The absorbance readings were measured at 760 nm using a microplate reader (Infinite 200, Tecan Austria, Grödig, Austria). The TPC values were calculated using a gallic acid calibration curve (0 to 100 ppm) and are expressed in milligrams of gallic acid equivalents (GAE) per 100 g of dried matter (mg GAE/100 g). The calibration curve used can be found in Appendix Figure 1.

2.5 Crude protein analysis

Crude protein content analysis was carried out using the Kjeldahl method with the Kjelttec auto-analyser (Kjelttec™ 8400, FOSS, Denmark). Two catalyst Kjeltabs, 12 mL of 98% concentrated sulfuric acid and 200 (±1) mg of the samples were added to 250 mL Kjeldahl tubes each. The contents of the tubes were digested at 420 °C for 60 min using the Kjelttec digestion unit. After digestion, the tubes were cooled for 10-20 min before loading into the auto-analyser unit, which then performs titration to a colorimetric endpoint. The crude protein content was calculated using the standard conversion factor of 6.25.

2.6 Dietary fibre analysis

The insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) were determined using Megazyme's Total Dietary Fiber (TDF) assay kit (Megazyme, Ireland). The method used to determine IDF and SDF were from Megazyme, based on the American Association of Cereal Chemists (AACC) soluble/insoluble dietary fibre method (for oat products), 32-21.01 and the AACC TDF method, 32-05.01.^[28] However, some small modifications were made to the procedure in order to separate the TDF samples into its constituent IDF and SDF. They include centrifugation to isolate the IDF residues, precipitation of SDF with 95% ethanol (v/v), followed by centrifugation to separate the SDF residues. 1.000 (±0.005) g of duplicates for each sample were treated enzymatically and sequentially to α-amylase, protease and amyloglucosidase. One duplicate was analysed for ash, while the other was analysed for protein using the Kjeldahl method as described in 2.5. The IDF and SDF were obtained using Equation 1 and Equation 2.

$$\text{Dietary fibre (\%)} = \frac{\frac{R1 + R2}{2} - p - A - B}{\frac{m1 + m2}{2}} \times 100$$

Equation 1

where $m1$ is the sample weight of duplicate 1, $m2$ is the sample weight of duplicate 2, $R1$ is the residue weight from $m1$, $R2$ is the residue weight from $m2$, p is the protein

weight of $R2$, A is the ash weight of $R1$, and B is the value obtained from Equation 2 below.

$$B = \frac{\frac{BR1 + BR2}{2} - BP - BA}{2}$$

Equation 2

where B is the blank value, $BR1$ is the blank residue weight of blank duplicate 1, $BR2$ is the blank residue weight of blank duplicate 2, BP is the protein weight of $BR2$, and BA is the ash weight of $BR1$.

2.7 Bread roll formulation

The bread roll formulation and bread-making procedure (see 2.8) was optimised in previous experiments with the goal of balancing between desirable sensory attributes while keeping the highest percentage substitution of unfermented and fermented BSG. The preliminary studies included tests of 10 and 15% BSG flour substitution. Table 1 shows the final bread formulations of the control bread roll (CTRL), 10% unfermented BSG bread roll (UFBSG10), and 10% Shōchū Black Koji fermented BSG bread roll (BKBSG10).

Table 1: Bread roll formulations (% based on flour weight)

	CTRL	UFBSG10	BKBSG10
Bread flour (%)	100	90	90
BSG (%)	0	10	10
Water (%)	83	83	83
Yeast (%)	3	3	3
Sea salt (%)	4	4	4

2.8 Bread-making procedure

The UFBSG used in baking was prepared as shown in 2.2, while the BKBSG was prepared as shown in 2.3, with a scale up. All fermentation conditions were kept the same, with a fermentation time of 4 days.

The CTRL, UFBSG10 and BKBSG10 dough were prepared by weighing out the dry ingredients into large mixing bowls. The dry ingredients were whisked before the addition of water at ambient temperature (28 °C). The ingredients were gently mixed until incorporated. The mixing bowls were then covered tightly with aluminium foil and left to rise at ambient temperature for 2 hours. After bulk fermentation was completed, the doughs were divided into 53 g portions and shaped into balls. The shaped doughs were then loosely covered and proofed for another 45 min, under the same conditions. The rolls were transferred onto a baking tray before immediately baked on the centre rack of a pre-heated oven (Electrolux, Stockholm, Sweden) at 250 °C (no fan) for 8 min. After 8 min, the rolls were considered partially baked. They were then cooled to ambient temperature, before storing in airtight containers for 13-15 hours.

To finish baking the rolls, they were transferred onto a baking tray before baking on the centre rack of a pre-heated oven (Tefal Delice Turbo, Haute-Savoie, France) at 210 °C (turbo) for 6 min. After fully baking the rolls, they were cooled to ambient temperature and kept in airtight containers for 2-3 hours before serving in the sensory evaluation. All bread rolls used in this study have been partially baked for freshness and logistical reasons with regards to the evaluation.

2.9 Sensory evaluation

The fermented BSG used in this work was deemed a non-novel food item by the Singapore Food Agency (SFA), due to the history of safe use and processing of *koji* and barley.^[29] As a result, the bread samples used in this study meet the exempt criteria of Nanyang Technological University's (NTU) Exempt Category 6, in which case, research on consumer acceptance studies may be carried out.

Through a prior screening, all selected panelists were aged 18 and above and were regular consumers of bread products (at least once a month). They have not participated in any food and/or beverage studies in the past 6 months and neither have diabetes nor any medical-related dietary restrictions. In addition, they have not had gastric bypass surgery and neither smoke nor are ex-smokers. For females only, no panelists were pregnant or nursing.

23 untrained consumer panelists participated in this sensory evaluation. They consisted of 56.5% women and 43.5% men. Three bread roll samples (CTRL, UFBSG10 and BKBSG10) were presented to the panelists for evaluation, which were prepared as described in **2.7** and **2.8**. The panelists were asked to complete a sensory evaluation questionnaire (see Appendix Figure 5). The samples were evaluated based on likeness of appearance, aroma, texture, taste and overall liking, following a 9-point hedonic scale (9 – like extremely; 1 – dislike extremely).^[30]

The samples were also evaluated using a 5-point Just-About-Right (JAR) scale, on colour (5 – much too dark; 3 – just about right; 1 – much too light), firmness (5 – much too firm; 1 – much too soft), grittiness (5 – much too gritty; 1 – much too fine), moisture (5 – much too moist; 1 – much too dry), saltiness (5 – much too salty; 1 – not at all salty enough) and aftertaste (5 – very strong and pleasant; 1 – very strong and unpleasant), where applicable.^[30] The full ratings and liking scores for both 9-point hedonic and 5-point JAR scales can be found in Appendix Figure 4.

This evaluation was conducted in a monadic sequential design, whereby the panelists were given one sample at a time and provided their feedback sequentially as well. Between each sample, breaks of 1-2 min were given to the panelists. The sample orders were randomised and counter balanced, with each serving sequence appearing an equal, or close to equal, number of times. Serving temperature was kept constant, with all samples microwaved for 30s (Panasonic, Osaka, Japan) prior to serving. All panelists cleansed their palates with table water crackers and water before the session and between samples. The sessions were conducted in a large, quiet, odour-free, classroom style arrangement (see Appendix Figure 2). The set-up for the panelists can be found in Appendix Figure 3.

2.10 Statistical analyses

All experiments and measurements were performed in triplicates. All data are presented as mean $\pm$ standard deviation. The Student's *t* test was used to calculate the means with significant differences. The differences were considered significant when $p < 0.05$.

Chapter 3 Results and Discussion

3.1 Determination of optimal *koji* starter

8 different *koji* starters were first investigated to determine which could bring about the largest nutritional improvement in BSG. The total phenolic content (TPC) of day 4 fermented BSG and unfermented BSG (UFBSG) are found in Figure 1.

Figure 1: Total phenolic content (TPC) of day 4 unfermented BSG (UFBSG) and day 4 fermented BSG using koji starters NJK110 (NJK), Shōchū Black Koji (BK), Fuji (FUJ), *Aspergillus oryzae* Hashimoto (AOH), SP-05 (SP5), EM-2 (EM2), SP-01 (SP1) and M-1 (M-1). All data are expressed as mean $\pm$ standard deviation ($n = 3$), different letters mean statistical difference ($p < 0.05$).

TPC had been selected as the determining parameter as the values have been shown to increase alongside other desirable parameters, such as soluble protein content and DPPH radical scavenging activity.^[31] From the above, day 4 M-1 fermented BSG has shown the greatest increase from 141 ± 5 mg GAE/100 g in UFBSG, to 954 ± 93 mg GAE/100 g. Additionally, its TPC is statistically different from all other fermented BSG samples. As a result, M-1 has been selected as the optimal *koji* starter for the fermentation of BSG.

3.2 Fermentation of BSG using M-1

Preliminary studies conducted for this work found that the factors affecting fermentation of BSG with *koji* starters include substrate particle size, moisture content, inoculum density, aeration and agitation, temperature, pH, humidity and fermentation time, matching what was observed in the literature.^[15] As a result, these factors were either optimised or fixed when performing experiments.

The fermentation of BSG using the selected *koji* starter M-1 was studied across 7 days using the parameters: TPC, crude protein and dietary fibre.

3.2.1 Total phenolic content

The total phenolic content (TPC) of M-1 fermented BSG (M1BSG) and UFBSG from day 0 to 7 are as shown in Figure 2.

Figure 2: TPC of M-1 fermented BSG (M1BSG) and UFBSG from day 0 to 7. All data are expressed as mean $\pm$ standard deviation ($n = 3$), different letters mean statistical difference ($p < 0.05$).

From the above, we observe that as fermentation time increases, the standard deviation of UFBSG increases. This can be attributed to the undesirable microbial activity from the environment, which is a trend that has been observed from preliminary studies. Hence, the preferred fermentation time of BSG would be before day 7, as a significant increase is observed between day 6 and 7, indicated by the different letters.

The general trend of the TPC of M1BSG is an increase from day 0 to 4, followed by a plateau. Given that fermentation time should be minimised to decrease undesirable microbial activity, day 4 is the optimal fermentation time for M1BSG.

A small observation to note is the TPC of day 0 UFBSG being significantly lower than that of day 1 to 6. The day 0 UFBSG samples were taken directly after pre-treatment as described in 2.2, while all other samples went through secondary grinding after SSF as described in 2.3. Secondary grinding decreases particle size, which in turn affects the TPC detected in the assay.^[32]

3.2.2 Crude protein content

Figure 3: Crude protein content of M-1 fermented BSG (M1BSG) from day 0 to 7. All data are expressed as mean $\pm$ standard deviation ($n = 3$), different letters mean statistical difference ($p < 0.05$).

Figure 3 shows the crude protein content of M1BSG across 7 days. The general trend observed is an increase in crude protein from day 0 to 4, followed by a plateau. This trend is the same as that of the TPC graph in Figure 2. Hence, day 4 is selected as the optimal fermentation time for M1BSG based on the TPC and protein graphs.

3.2.3 Dietary fibre

With the optimal fermentation time selected, day 4 M1BSG was analysed for its IDF and SDF, alongside control UFBSG as shown in Figure 4.

Figure 4: Insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) of M-1 fermented BSG (M1BSG) and unfermented BSG (UFBSG). All data are expressed as mean $\pm$ standard deviation ($n = 3$), different letters mean statistical difference ($p < 0.05$).

Here, we see that the IDF of the samples were not statistically different, as were the SDF. Day 4 M1BSG was observed to have lost $17.0 \pm 1.0\%$ of its dry mass after fermentation. Knowing this, if M-1 does not produce cellulase, we can expect the IDF and SDF of BSG to increase in dry weight concentration after fermentation. However, with the results as shown, we can thus conclude that M-1 does possess some level of cellulase activity.

3.3 Sensory Evaluation

The main goal of this sensory evaluation is to better understand the market value and identify possible trends on unfermented and fermented BSG-fortified bread. Bread rolls were selected for the sensory evaluation as cereal-based food is considered to be the most suitable for BSG substitution, since BSG is also derived from cereal whole grains.

The *koji* starter Shōchū Black Koji was used to ferment BSG rather than M-1 due to the taste of the fermented material. The latter was very distinct in flavour, whereas Shōchū Black Koji fermented BSG (BKBSG) was much more neutral and was hence expected to be easier to incorporate in bread.

3.3.1 Characteristics of bread rolls

The three bread rolls used in this sensory evaluation were the control bread roll containing 0% BSG (CTRL), 10% substitution of bread flour using unfermented BSG (UFBSG10), and 10% substitution of bread flour using day 4 Shōchū Black Koji fermented BSG (BKBSG10). The nutritional composition and appearance of the bread rolls are as shown in Table 2 and Figure 5, respectively.

Table 2: Nutritional composition of bread rolls (hydrated). LOD, limit of detection.

	LOD	CTRL	UFBSG10	BKBSG10
Protein (g/100g)	0.1	9.9	9.7	10.7
Carbohydrates* (g/100g)	0.1	47.7	44.1	46.8
Fat (g/100g)	0.05	1.44	1.75	2.01

*Including total dietary fibre (TDF).

The results reported have been performed by Australian Laboratory Services (ALS), in accordance with the terms of accreditation under the Singapore Accreditation Council.

From Table 2, UFBSG10 was found to have a slightly lower protein content than CTRL. Bread flour itself is high in protein, making the two bread rolls comparable in protein content. However, a significantly higher protein content of BKBSG10 than that of UFBSG10 was observed, which can be attributed to fermentation.

The carbohydrate content, including TDF, was observed to be lowest in UFBSG10, followed by BKBSG10 and CTRL. While simple carbohydrates are generally worse for human health, a high dietary fibre content and complex carbohydrates from whole grains are extremely important to human health.^[10, 33] As a result, more information in terms of TDF data and carbohydrate composition would be needed in order to draw a conclusion.

The fat content is found to be lowest in CTRL, followed by UFBSG10 and BKBSG10. From this, we are able to infer that the fat content has slightly increased after fermentation, and that the fat content of UFBSG is higher than that of bread flour.

Figure 5: Appearance of bread samples from top-down (first row) and cross-sectional (second row).

3.3.2 9-point hedonic scale

23 untrained consumer panelists (aged 18 and above) were selected for this sensory evaluation, among which 56.5% women and 43.5% men. The results of the 9-point hedonic scale are as shown in Table 3.

Table 3: Results on 9-point hedonic scale

	CTRL	UFBSG10	BKBSG10
Overall liking	6.87 ± 1.52 ^b	5.83 ± 1.90 ^a	5.04 ± 2.12 ^a
Appearance	6.96 ± 1.92 ^b	5.17 ± 1.99 ^a	5.57 ± 1.70 ^a
Aroma	6.39 ± 1.64 ^b	6.13 ± 1.55 ^b	5.04 ± 2.06 ^a
Texture	6.96 ± 1.72 ^b	5.65 ± 2.08 ^a	5.48 ± 2.04 ^a
Taste	6.78 ± 1.54 ^c	5.87 ± 1.96 ^b	4.78 ± 2.07 ^a

All data are expressed as mean ± standard deviation, with the different lowercase letters following the values meaning statistical difference ($p < 0.05$).

From the above, we see that overall liking of UFBSG10 and BKBSG10 were significantly lower than that of CTRL. Healthier products tend to have poorer consumer acceptance compared to commonly consumed food such as bread. Given that the BSG-fortified samples were healthier, the findings were hence not unexpected. For the external appearance, UFBSG10 and BKBSG10 were also significantly lower than that of CTRL. It is obvious to assume that the lower ratings on appearance were due to colour, however, the same trend was not observed for the colour JAR (see Table 4). This trend can thus only be explained through follow-up questions.

The aroma of UFBSG10 and CTRL had no significant differences, making them equally well-liked. However, the aroma of BKBSG10 was found to be significantly lower. This was likely due to generation of volatile compounds during fermentation^[34] which formed a characteristic aroma for BKBSG, making it less desirable to the panelists. For texture, both UFBSG10 and BKBSG10 were significantly lower than CTRL. This trend is similarly observed in the JAR ratings for grittiness (see Table 4). Thus, it is likely that the texture ratings were lower due to higher grittiness. Studies on the sensory evaluations of BSG-fortified food reported similar findings as well.^[23]

Lastly, the taste of UFBSG10 was significantly lower than that of CTRL. This was likely due to the healthier whole grains in UFBSG10 that led to its poorer consumer acceptance. Additionally, the taste of BKBSG10 was observed to be significantly lower than UFBSG10. In the sourness JAR (see Table 4), BKBSG10 was significantly higher than UFBSG10 and CTRL. The undesirable sourness in combination with the characteristic taste of BKBSG10 were hence likely the reasons for poorer taste ratings.

3.3.3 5-point Just-About-Right scale

The results for the 5-point JAR scale on colour, firmness, grittiness, moisture, saltiness, sourness, and aftertaste are shown in Table 4.

Table 4: Results on 5-point Just-About-Right (JAR) scale

	CTRL	UFBSG10	BKBSG10
Colour	2.43 ± 0.66 ^a	3.09 ± 0.90 ^b	3.74 ± 0.54 ^c
Firmness	3.17 ± 0.72 ^a	3.09 ± 0.73 ^a	2.91 ± 0.42 ^a
Grittiness	3.13 ± 0.81 ^a	3.48 ± 0.79 ^{ab}	3.65 ± 0.88 ^b
Moisture	3.17 ± 0.72 ^a	3.30 ± 0.70 ^a	3.09 ± 0.79 ^a
Saltiness	2.83 ± 0.72 ^a	2.91 ± 1.00 ^a	2.91 ± 0.90 ^a
Sourness	3.26 ± 0.45 ^a	3.13 ± 0.55 ^a	3.74 ± 0.81 ^b
Aftertaste	3.32 ± 0.58 ^b	2.84 ± 0.76 ^a	2.55 ± 1.14 ^a

All data are expressed as expressed as mean ± standard deviation, with the lowercase letters following the values meaning statistical difference ($p < 0.05$).

From the above, the colour of UFBSG10 was closest to just-about-right, followed by CTRL (lighter) and BKBSG10 (darker), all of which were statistically different. The fermentation of BKBSG breaks down complex macromolecules, bringing about changes in chemical composition. As a result, pyrolysis while baking may have occurred at a faster rate, leading to a darker outer appearance of BKBSG10.

The grittiness of UFBSG10 was not significantly different from CTRL. This meant that a 10% substitution of UFBSG was equally accepted. However, BKBSG10 grittiness was observed to be significantly greater than CTRL. This was an unexpected finding, as the particle sizes of BKBSG (88- 595 μm) tended to be smaller than UFBSG (177-595 μm). It is possible that the inclusion of more panelists may be required for more accurate observations on grittiness.

The sourness of UFBSG10 and CTRL were close to just-about-right, while that of BKBSG10 was significantly greater. This was expected as fermentation could potentially increase the acidity of its substrates.^[35] For the aftertaste JAR, UFBSG10 and BKBSG10 were significantly lower than CTRL. The same trend was observed in the 9-point hedonic taste ratings (see Table 3). The results from the aftertaste JAR can likely be explained similarly.

For the last few sensory characteristics of firmness, moisture and saltiness, the samples were observed to be not statistically different from one another.

Chapter 4 Conclusion

In conclusion, the optimisation of the fermentation of brewer's spent grain (BSG) was investigated in order to enhance its nutritional profile. M-1 was selected as the optimal *koji* starter for the fermentation of BSG, with a fermentation time of 4 days. Through M-1 fermentation, the nutritional profile of BSG improved in terms of total phenolic content as well as crude protein content.

In addition, the nutritional profile and sensory characteristics of 10% substituted unfermented BSG (UFBSG10) and Shōchū Black Koji fermented BSG bread rolls (BKBSG10) were investigated. Further analyses on total dietary fibre content, quality of protein as well as carbohydrates are required to better understand the extent of nutritional enhancement from fermentation. Despite this, it was conclusive that BKBSG10 had an improved nutritional profile, given the increase in total phenolic content of day 4 BKBSG and protein content of BKBSG10. Both BSG-fortified bread rolls were healthier than CTRL, with the substitution of bread flour using whole grains.

BKBSG10 showed poorer consumer acceptability in aroma, taste, colour and sourness compared to UFBSG10. With that being said, BKBSG10 seems be somewhat controversial, as seen from its consistently large standard deviations, especially obvious in the 9-point hedonic results. Hence, there is potentially a niche market for fermented BSG food products, with many different flavour profiles yet to be discovered from using different food microbes. With this, fermentation could still potentially bridge the gap of BSG incorporation in food.

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Appendix

Appendix Figure 1: Gallic acid calibration curve for TPC calculation

Appendix Figure 2: Sensory evaluation session

Appendix Figure 3: Set-up for panelists

a)	Hedonic Rating	Liking Score
	Like Extremely	9
	Like Very Much	8
	Like Moderately	7
	Like Slightly	6
	Neither Like Nor Dislike	5
	Dislike Slightly	4
	Dislike Moderately	3
	Dislike Very Much	2
	Dislike Extremely	1
b)	Aftertaste JAR	Liking Score
	Very Strong And Pleasant	5
	Somewhat Strong And Pleasant	4
	Neither Pleasant Nor Unpleasant	3
	Somewhat Strong And Unpleasant	2
	Very Strong And Unpleasant	1
c)	Saltiness JAR	Liking Score
	Much Too Salty	5
	Somewhat Too Salty	4
	Just About Right	3
	Not Quite Salty Enough	2
	Not At All Salty Enough	1
d)	Sourness JAR	Liking Score
	Much Too Sour	5
	Somewhat Too Sour	4
	Just About Right	3
	Somewhat Too Sweet	2
	Much Too Sweet	1
e)	Firmness JAR	Liking Score
	Much Too Firm	5
	Somewhat Too Firm	4
	Just About Right	3
	Somewhat Too Soft	2
	Much Too Soft	1
f)	Grittiness JAR	Liking Score
	Much Too Gritty	5
	Somewhat Too Gritty	4
	Just About Right	3
	Somewhat Too Fine	2
	Much Too Fine	1
g)	Colour JAR	Liking Score
	Much Too Dark	5
	Somewhat Too Dark	4
	Just About Right	3
	Somewhat Too Light	2
	Much Too Light	1
h)	Moisture JAR	Liking Score
	Much Too Moist	5
	Somewhat Too Moist	4
	Just About Right	3
	Somewhat Too Dry	2
	Much Too Dry	1

Appendix Figure 4: Full 9-point hedonic scale (4a) and full 5-point Just-About-Right (JAR) scales for aftertaste (4b), saltiness (4c), sourness (4d), firmness (4e), grittiness (4f), colour (4g) and moisture (4h).

Sensory Evaluation Questionnaire (Sample #1)

You will be evaluating 3 samples of bread rolls in this session. You have just been served the first sample. Thinking specifically about the way this bread roll looks...

1. How much do you like or dislike the appearance of this bread roll? (*9-point hedonic*)
2. Would you describe the colour of this bread roll as... (*Colour JAR*)

To evaluate the aroma, please tear the roll in half and take a good sniff. Thinking specifically about the way it smells...

3. How much do you like or dislike the aroma of this bread roll? (*9-point hedonic*)

Before you taste this sample, take a sip of water and a bite of cracker to remove any lingering tastes in your mouth. Feel free to rinse your mouth with water whenever you need during this session. Please eat at least one-half of this bread roll so that you will be able to form an opinion.

4. Everything considered, how much do you like or dislike this bread roll overall? (*9-point hedonic*)

Thinking specifically about its texture... (Texture is the way food feels when you eat it)

5. How much do you like or dislike the overall texture of this bread roll? (*9-point hedonic*)
6. Would you describe the softness/firmness of this bread roll as... (*Firmness JAR*)
7. Would you describe the grittiness (sandy/coarse)/fineness of this bread roll as... (*Grittiness JAR*)
8. Would you describe the moisture/dryness of this bread roll as... (*Moisture JAR*)

Thinking specifically about the way it tastes...

9. How much do you like or dislike the overall taste/flavour of this bread roll? (*9-point hedonic*)
10. Would you describe the overall saltiness of this bread roll as... (*Saltiness JAR*)
11. Would you describe the sourness/sweetness of this bread roll as... (*Sourness JAR*)
12. Would you describe the aftertaste from this bread roll as... (*Aftertaste JAR, with 'none' option*)

Please raise your hand to indicate that you are done with this sample. You will now take a 1-2 min break before moving on to the next sample, please cleanse your palate in the meantime.