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Coláiste na hOllscoile Corcaigh

Fundamental study of the application of brewers spent grain and fermented brewers spent grain on the quality of pasta

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Abstract

Upcycling and repurposing of side streams from food processing have become a necessity to merge our world into a more sustainable future. Brewers spent grain (BSG) is a highly abundant and nutrient rich by-product of the brewing industry. The aim of this study was to investigate the effect of fermentation on BSG (FBSG) while also examining the effects of including fibre rich BSG and FBSG ingredients on techno-functional and nutritional properties of semolina-based pasta. The gluten network formation, starch gelatinisation, texture, cooking loss, optimal cooking time, *in vitro* starch digestibility and ultrastructure of the pasta was investigated. BSG and FBSG inclusion weakened gluten network properties versus the semolina control but was more favourable than the wholemeal control. Addition of BSG and FBSG produced pasta with a greater nutritional profile, having a higher fibre content and lower predicted glycaemic index compared to semolina pasta. BSG and FBSG addition enhanced tensile strength and pasta firmness versus wholemeal pasta. An increased reduction in the predicted glycaemic index was noted with FBSG inclusion at the higher level of addition compared to BSG, suggesting fermentation of BSG may further enhance nutritional properties of the BSG ingredient.

1. Introduction

Brewers' Spent Grain (BSG) represents approximately 85% of the total by-products produced from brewing. Following beer production, on average, about 100 kg-130 kg of wet BSG (water content of approx. 80%) is generated from 100 kg of malt (Kunze, 2004). The increase in mass compared to malt is due to the high water content of BSG. BSG has attracted considerable attention due to the vast quantities of waste associated with it. The current primary use of BSG is animal feed; however, increased awareness of the nutritional profile of BSG has sparked investigation of its potential use as a food ingredient (Mussatto, 2014).

BSG is a lignocellulosic material rich in cellulose, hemicellulose, lignin, proteins, minerals and a low level of fat (Lynch et al., 2016). The composition of BSG can vary. Variations in BSG composition may be due to differences in barley grain type; malt type; grain cultivation; brewing process and equipment; the stage in brewing at which BSG is collected; and the location at which the BSG sample is taken from the filter cake as protein, fat and fibre contents are not evenly distributed (Hennemann et al., 2019). In addition, some brewing processes may incorporate other cereal adjuncts within their process, and remnants of these adjuncts may also be present in BSG (Mussatto, 2014; Santos et al., 2003). However, fibre and protein are the predominant fractions in BSG (Lynch et al., 2016; Mussatto et al., 2006). Protein constitutes approximately 19-30% of BSG, while fibre (cellulose, hemicellulose, lignin) represents 30-50% of the BSG composition (Mussatto, 2014). The hemicellulose fraction of BSG is mainly comprised of arabinoxylans, which can be present at levels of up to 40%. The arabinoxylans consist of a xylose backbone with substituted arabinose residues and ferulic acid esterified to the arabinose residues (Lynch et al., 2016).

Evidence exists to link fibre consumption with helping in controlling body weight, type-2-diabetes, and possibly lowering the risk of developing some cancers and coronary heart disease (Kendall et al., 2010). With fibre holding a large proportion of the composition of BSG, it is of interest to incorporate into the human diet. Previous attempts have been made to incorporate BSG into food products, such as snack foods, bread and pasta (Ainsworth et al., 2007; Nocente et al., 2019; Stojceska et al., 2008; Waters et al., 2012). Improvements in nutritional profiles of foods have been noted, particularly in relation to the increase in fibre (Nocente et al., 2019).

Fermentation of foods and ingredients has previously enhanced features such as sensory, shelf life, functionality and nutritional properties (Hutkins, 2006; Sahin et al., 2019; Waters et al., 2013). Successes have been found with BSG and brewers spent grain sourdough supplemented in wheat bread, with BSG fortified breads showing more favourable outcomes than the wholemeal control (Waters et al., 2012). Fermentation improved textural properties of the bread and proved acceptable by a sensory panel up to a 10% addition level (Waters et al., 2012).

A review carried out by Lynch et al., (2016), highlights BSG as a suitable material for inclusion in cereal-based products while also being an attractive substrate for fermentation. The aim of this study was to determine the effects of fermentation on BSG at a molecular level and investigate the effects of the inclusion of BSG and fermented brewers spent grain (FBSG) ingredients in pasta formulations. Semolina and wholemeal flour were used as controls throughout the study. Analysis focussed on the effects of increasing fibre contents of pasta using BSG and FBSG ingredients; with ingredients added to pasta formulations according to Regulation (EC) No 1924/2006 (EU, 2006), where fibre levels present in the final pasta product had 3 g/100g (Source of Fibre) and 6 g/100g (High in Fibre).

2. Experimental

2.1 Raw Materials

Semolina (East End Foods PLC, West Bromwich, UK) and stone grinded Wholemeal flour (WM) (Odlum Group, Dublin, Ireland) were used as control flours for this experiment. Salt (Glacia British Salt Limited, Cheshire, UK) and tap water were also incorporated into pasta recipes. Milled and spray-dried BSG and FBSG were produced and provided by Anheuser-Busch (Anheuser-Busch InBev, Leuven, Belgium). FBSG was produced according to patent number WO/2018/033521 (Gil-Martinez & Arendt, 2018).

2.2 Compositional Analysis of Raw Materials

Compositional analysis for semolina, WM, BSG and FBSG were performed by Concept life Sciences Ltd (Bar Hill, UK). Protein was determined using the Dumas principle (conversion factor= 6.25); moisture was evaluated using oven drying (105 ^{o}C) for a minimum of 16 h; fat was determined using low resolution proton nuclear magnetic resonance; ash content was calculated by oxidation at 550 ^{o}C to remove organic matter, leaving the mineral residue. Total carbohydrates were calculated by difference; sugars were determined on hot water extraction of the sample by ion chromatography with pulsed amperometric detection using a gold electrode and a calibration against an internal standard. Dietary fibre values for semolina and wholemeal flours were analysed in accordance with AOAC method 991.43. The dietary fibre values for BSG and FBSG were provided by the supplier, Anheuser Busch (Leuven, Belgium) and were determined according to AOAC method 2011.25**.** Digestible and resistant starch values of the ingredients were measured using the Megazyme kit K-RAPRS (Bray, Ireland). Total starch was calculated as the sum of digestible starch and resistant starch. Starch analysis was performed on cooked freeze-dried pasta and calculated based on moisture content of cooked vs freeze-dried pasta samples.

2.3 Alpha-amylase and Beta-amylase activity of fibre ingredients

The alpha-amylase activity of the ingredients was determined using the alpha-amylase assay kit (ceralapha method) supplied by Megazyme (Bray, Co. Wicklow, Ireland). Beta-amylase activity was determined using K-BETA3 assay kit also supplied by Megazyme.

2.4 Protein Profile Analysis

The protein profile of BSG and FBSG were analysed to investigate the effect of the fermentation process on proteins present in BSG. An Agilent Bioanalyzer 2100 Lab-on-a-Chip capillary electrophoresis system was used to analyse the protein profile and estimate molecular weights of the samples. Samples were prepared according to Amagliani et al., (2017), with slight modifications: ingredients were dispersed in 2% SDS, 2 M thiourea, and 6 M urea, to give a protein concentration of 2% w/v. Dispersions were shaken for 2 hours at room temperature and then centrifuged to remove insoluble material. Samples were analysed using an Agilent Protein 80 kit and Protein 230 kit according to the instructions within the ranges of 5–80 and 14–230 kDa, respectively. The protein 230 kit did not show any differences; hence data not shown. For stronger reducing conditions, Dithiothreitol (DTT) was included in the sample buffer according to kit instructions.

2.5 Addition levels of the fibre ingredients to pasta formulas

Inclusion of fibre was adjusted in accordance with “source of fibre” (SF) and “high in fibre” (HF) claims (EU, 2006), referring to cooked pasta. The claim applies to the final food product; therefore BSG and FBSG were adjusted with uptake of water by the pasta during cooking considered. Water uptake was calculated by determining the difference in moisture content between raw and cooked pasta formulations. Moisture was measured using Moisture Analyser LJ16 (Mettler Toledo, Ohio, US). Fibre ingredient additions (Table 1) were calculated based on the water taken up and adjusted to reach 3g/100g and 6g/100g claims.

2.6 Impact of fibre ingredients on gluten network

Analysis of gluten aggregation in the flours was investigated using GlutoPeak (Brabender GmbH and Co KG, Duisburg, Germany). 9 g of sample (based on 14% moisture) was added to deionised water (36 ^{o}C) to a total volume of 18 g in the device sample cup. Flour blends endured a hand premixing step to ensure a homogenous blend was added to the deionised water. The sample slurries were subjected to high shear (2750 rpm: 36 ^{o}C).

2.7 Effect of fibre ingredients on starch pasting properties

Pasting temperature, peak viscosity, final viscosity and breakdown values were measured using a Rapid Visco Analyser (RVA Super 3, Newport Scientific, Warriewood, Australia). Three grams of the solid sample (based on 14% moisture) was added to deionised water to a total

volume of 28 g. Flour blends were premixed before addition to water. The samples were mixed at a constant shear rate (160 rpm), and a temperature profile was applied as reported by Horstmann et al., (2017).

2.8 Pasta Preparation

Recipes for pasta production are illustrated in Table 1. For each formulation, a total dough volume of 1 kg was prepared. Dry ingredients were premixed using a Kenwood chef mixer (Kenwood Ltd., New Hampshire, UK) with a K-beater for 2 mins. An adjusted volume of tap water (30 ^{o}C) was added and mixed for 10 mins. For fibre enriched recipes, the amount of water added was adjusted by adding water at different levels to obtain an optimal crumbly dough consistency. The dough was transferred to a single screw extruder (PN 300 extruder, Haussler, Heiligkreuztal, Germany) equipped with a spaghetti die (internal diameter 2mm). Pasta samples of a length of 20 cm were produced. Fresh pasta was used in the analysis.

2.9 Pasta Characterisation

Analysis of each batch of fresh pasta was conducted on the same day of production.

2.9.1 Optimal Cooking Time

Optimal cooking time (OTC) is the time (mins) it takes for the core of the spaghetti strand to gelatinise fully. OTC is measured as the time it takes for the spaghetti core to become opaque when pressed between two glass slides and was determined according to AACC Approved Method 16–50 (AACC International, 1995), as reported by Hager et al., (2012). This was performed before texture parameters of the pasta were analysed.

2.9.2 Cooking Loss

Cooking loss (CL) indicates the content of dry matter lost from the pasta during cooking, with a low cooking loss desired. This was determined using AACC Approved Method 16–50, as previously reported by Hager et al., (2012).

2.9.3 Texture properties of cooked pasta

Firmness, tensile strength and stickiness were analysed on cooked pasta using a TA.XT*plus* texture analyser (Stable Micro Systems, Godalming, Surrey, UK) set with a 5 kg load cell.

The pasta firmness represents the resistance the pasta strand exhibits to a force and indicates the degree of the “al dente” mouthfeel. Firmness was determined according to the AACC

spaghetti firmness method 66-52.01 and expressed as max cutting force (N). Firmness of the pasta was determined using the heavy-duty platform with a light knife blade and transparent Perspex plate (Stable Micro Systems, Godalming, Surrey, UK). Five spaghetti strands were aligned parallel on the centre of the texture analyser platform with a perspex blade attached. A trigger force of 0.05 N, test speed 0.17 mm/sec and a 4.5 mm distance were the testing parameters used. The test was repeated five times for each pasta batch produced.

Tensile strength reveals the elasticity of pasta strands and is defined as the resistance to uniaxial extension (expressed as maximum breaking strength). This was measured using the tension test A/SPR spaghetti/noodle tensile rig with a trigger force of 0.05 N, a test speed of 3 mm/sec and a 100 mm distance (Stable Micro Systems, Godalming, Surrey, UK). The analysis was performed on 10 strands of pasta strands (10 cm) per batch.

Pasta stickiness is an indication of the cooking quality of pasta, with excessive stickiness being undesired. It is defined as the max peak force (N) when the probe is retracted from the sample and was recorded using the pasta stickiness rig (HDP/PFS, Stable Micro Systems, Godalming, Surrey, UK). Five spaghetti strands were aligned in the centre of the raised platform of the texture analyser, under a rectangular aluminium probe, held by a plate with a rectangular opening. Test parameters included a trigger force of 0.2 N, test speed 0.5 mm/sec and distance of 25 mm. The analysis was repeated 10 times per batch produced.

2.9.4 Scanning Electron Microscopy (SEM)

Freeze-dried pasta was mounted on stubs (G 306; 10 mm x 10 mm Diameter; agar scientific, UK) and fixed using carbon tape (G3357N; Carbon Tabs 9 mm; agar scientific, UK). Mounted pasta samples were sputter coated with a gold-palladium alloy (ratio of 80/20), using a Polaron E5150 sputter coating unit, and imaging was captured with a JEOL Scanning Electron Microscope (JSM-5510, Jeol Ltd., Tokyo, Japan). Settings were implemented as follows: 5 kV voltage, 20 mm working distance and a magnification factor of 1000.

2.10 *In vitro* starch digestibility as an indication of glycaemic index

In vitro starch digestibility determination is based on enzymatic degradation of digestible starch to reducing sugars over time.

An *in vitro* digestion assay for fibre enriched products was conducted as reported by Brennan & Tudorica (2008). Samples endured proteolytic treatment using a pepsin solution, followed by a 5 h incubation with pancreatic α-amylase solution within a dialysis tube. The amount of reducing sugars (maltose) released from the dialysis tubing system into the buffer was determined spectrophotometrically using 3,5-dinitrosalicylic acid (DNS) solution. Samples were taken every 30 min. 100 µl DNS was added to 100 µl of the sample taken, heated on a dry heating block at 100 °C for 15mins and diluted with 1 ml of distilled water. The absorbance at wavelength 546 nm was determined. All analysis was completed in duplicate. The reducing sugar release (RSR); the maltose diffusion in presence of the sample (DIFF sample); and the sugar diffusion index (SDI) were determined as reported by Brennan & Tudorica (2008). The predicted glycaemic index (pGI) was calculated using the following formula:

$$GI_{predicted} = 105.52 \times \frac{Fibre}{Carbohydrate} - 76.46 \times \frac{protein}{carbohydrate} + 1.23 \times RSRI_{at150\,min} + 69.41 \times SDI_{at\,270\,min} - 83.87$$

2.11 Statistical Analysis

All experimental analysis was carried out in triplicate unless stated otherwise. One-way ANOVA test using a Tukey test ($p \leq 0.05$) was performed using Minitab version 19 (Minitab LLC., State College Pa.). Correlation analysis was carried out using Microsoft Excel.

3. Results & Discussion

3.1 Compositional Analysis of main ingredients used.

Results from compositional analysis of the ingredients used in the analysis are represented in Table 2.

3.1.1 Protein content

Protein content for semolina was 13.2%. Semolina (made from durum wheat) is the preferred raw material for pasta making, and protein levels measured for semolina in this study were similar to previous findings (Boyacioglu & D'Appolonia, 1994; Petitot et al., 2010). WM flour had a lower protein content (11.4%), which could be attributed to differences in wheat variety or cultivar (Davis et al., 1981; Khan & Shewry, 2009). . BSG contained 31.4% protein. BSG is naturally high in protein, and levels of protein measured in this study were similar to protein concentrations previously reported (Table 2). The protein content of FBSG (32.4%) was slightly higher than the protein level measured in BSG and could be linked with the combined effect of batch variations of BSG and potential differences in the point at which the BSG sample was collected from the filter cake, as protein contents can vary within the filter cake (Hennemann et al., 2019). However, the difference in protein concentration was minimal and was comparable with the level of protein expected (Table 2).

3.1.2 Protein profile

Figure 1 illustrates the results of the protein profile analysis of BSG and FBSG. The main proteins found in BSG are hordeins (Celus et al., 2006). The hordeins may be separated into subunits, A hordeins <20kDa, B hordeins 35-50kDa, C hordeins 55-80kDa and D hordeins 96kDa based on previous publications (Celus et al., 2006; Howard et al., 1996; Shewry et al., 1977). Differences were observed in the size of the proteins present in BSG versus FBSG; indicating the fermentation process influenced the protein profile. FSBG contained a greater amount of low molecular weight proteins (particularly in the range of ~ 15 – 46 kDA) than BSG, likely due to proteolysis during the fermentation process. DTT addition induces stronger reducing conditions to ensure breakup of inter/intra disulphide bonds in proteins. DTT addition for BSG and FBSG resulted in higher amounts of low molecular weight protein versus without DTT addition, indicating BSG and FBSG proteins consist of smaller subunits. The enhanced luminous intensity in the lower region of FBSG with DTT addition indicates a greater

number of smaller molecular weight proteins were present after fermentation and may be influencing outcomes in gluten network formation discussed in later sections (section 3.2).

3.1.3 Minerals

Ash content for WM flour and semolina were 1.3% and 1%, respectively. Higher levels of minerals were present in BSG (3.7%) and FBSG (3.7%). BSG and FBSG are comprised of the outer layers of the barley grain (pericarp, seed coat and husk material), where minerals are concentrated in grains, hence the high levels present (Arendt & Zannini, 2013). Levels of minerals present in BSG were in line with literature values for BSG (Table 2). Fermentation of BSG had no effect on mineral contents. However, some of the minerals in FBSG may be more bioavailable post fermentation (Poutanen et al., 2009). Lactic acid bacteria produce lactic acid during fermentation, which creates an acidic environment and enhances phytase activity. This contributes to the reduction of phytates, making more minerals available for absorption (Lopez et al., 2001, 2003; Poutanen et al., 2009).

3.1.4 Fat

Semolina had the lowest fat content (1.3%) followed by WM flour (1.6%). Fat contents of BSG and FBSG were 10.3% and 6.53% respectively. Lipid content for BSG and FBSG were within range of previously reported values for BSG (Table 2). The variances in fat content observed between BSG and FBSG could be due to batch to batch variations in the brewing process and potential differences in BSG sample collection from the lauter tun. Lipid contents can be inhomogeneous within the filter cake in brewing and may account for the differences in fat observed (Hennemann et al., 2019).

3.1.5 Carbohydrates

3.1.5.1 Sugars

Sugar levels reported for semolina and WM were 1.4% and 1.2%, respectively. Sugar levels in BSG (0.2%) were low. Sugars are lost to the wort during the mashing process in brewing (Pires & Bránayik, 2015); hence the very low levels found**.** An increased level of sugars was reported for FBSG (2.9%) in comparison to BSG (0.2%), which may be linked to the combined hydrolysis and fermentation process employed for FBSG production. Fibres and starch are degraded during this process, which liberates small chain polysaccharides and monosaccharides (Mussatto et al., 2008; Xiros & Christakopoulos, 2012)

3.1.5.2 Dietary Fibre

Semolina contained 5% dietary fibre, while WM flour had a dietary fibre value of 7.1%. WM flour contains higher levels of dietary fibre than semolina due to the increased prevalence of the bran and germ layers in the flour. Dietary fibre levels in BSG (42.6%) and FBSG (49.4%) were significantly higher than the control flours. BSG is naturally high in fibre, namely insoluble fibre (Waters et al., 2012), with arabinoxylans being the predominant fibre present (Cui et al., 2013; Lynch et al., 2016). A higher dietary fibre content was observed in FBSG (49.4%) than BSG (42.6%). The differences observed in dietary fibre content may be attributed to batch variation of BSG or potential differences in BSG sample collection (Hennemann et al., 2019). However, the combination of microbial enzymes and the mixture of enzymes added to FBSG may solubilise some dietary fibre in FBSG vs BSG, particularly in relation to the arabinoxylans (Katina et al., 2007).

3.1.5.3 Starch Analysis

Total starch levels are reported for semolina and WM in Table 2. Starch levels in BSG (2.31 $\pm$ 0.05%) and FBSG (3.75 $\pm$ 0.06%) were much lower. BSG consists of the outer layers of the barley grain which include minimal levels of starch (Lynch et al., 2016). Starch is lost to the wort during mashing; therefore, a low level of starch was expected. Starch values recorded were in line with previous findings for BSG in Table 2 (Lynch et al., 2016). A high proportion of the starch present in BSG and FBSG was resistant starch. The term resistant starch refers to the starch, which is not broken down in the small intestine but rather slowly fermented in the large intestine. In BSG, 41.9% of the total starch was resistant starch, while in FBSG, 33.9% of the total starch was resistant starch. Variances in starch levels observed in the BSG ingredients could be linked with diversities found in BSG composition due to sample collection (Hennemann et al., 2019) as well as batch variations of BSG.

3.1.6 Alpha and Beta amylase results

Alpha and beta-amylase activities for semolina and WM are outlined on Table 2. The slightly higher amylase activity in semolina vs WM flour could be linked with some sprouting occurring which tends to increase amylase activity (Sissons et al., 2012). Minor differences in beta amylase activity indicated the fermentation did not have a major impact on residual beta-amylase activity. FBSG contained almost double the amount of alpha-amylase (0.24 $\pm$ 0.00 CU/g) than BSG (0.12 $\pm$ 0.00 CU/g). This is likely due to the addition of alpha-amylases

during the fermentation process and the potential production of amylases from lactic acid bacteria during fermentation (Padmavathi et al., 2018).

3.2 Impact of fibre ingredients on gluten network development

The incorporation of fibre-rich ingredients affected the torque maximum (TM) and the peak maximum time (PMT) of the gluten network development in semolina-based pasta (Figure 2).

Comparing the controls with each other, semolina showed a continuous increase in torque reaching a TM at 45 BU after 91.3 $\pm$ 0.6 sec, while WM resulted in a slower increase in average torque with a TM at 27.7 $\pm$ 1.2 BU after 126 $\pm$ 7.5 sec. The significantly weaker gluten network occurred due to the presence of coarse bran particles which interfered with the gluten network development (Noort et al., 2010; Wang et al., 2003; Wang et al., 2004).

The replacement of semolina with BSG and FBSG to achieve SF claim led to a significantly faster and stronger gluten network development than the semolina control, with FBSG causing the fastest development (65.3 $\pm$ 6.1 sec). Furthermore, an increase in TM was observed in samples including BSG (52 $\pm$ 1 BU) and FBSG (52.3 $\pm$ 0.6 BU). The ingredients BSG and FBSG contain a significant amount of proteins (Table 2), which amplified the development of a protein network, mainly by their charged amino acids (Waters et al., 2012), resulting in a stronger network. Moreover, BSG and FBSG are rich in minerals (Table 2). Minerals induce a charge screening effect and the exposure of apolar protein residues, which causes stronger hydrophobic interaction in the protein and leads to increased aggregation (Belz et al., 2012).

The higher addition level of fibre ingredients resulted in curves which were not comparable with any of the control flours. The inclusion of BSG at HF level showed a pronounced peak after 21 sec, followed by a steady torque at 45 BU. The ratio of glutenin's to gliadins is known to be a factor in determining the strength of gluten network (Edwards et al., 2003). Semolina flour from durum wheat contains a higher proportion of gliadins, which results in a slightly weaker gluten network (Boyacioglu & D'Appolonia, 1994; Huebner & Wall, 1976). Melnyk et al. (2012) reported an increase in gluten strength with increasing levels of glutenin inclusion. The inclusion of BSG at the higher level of addition is likely to be shifting the balance of glutenin and gliadins present, enhancing the glutenin proportion and causing an increase in torque.

The replacement of semolina by FBSG resulted in two peaks at the HF addition level. An initial torque of 43 BU was reached after around 21 seconds, followed by a TM of 50.3 ± 0.6 BU after 45 ± 3 seconds. The presence of two peaks indicates additional protein aggregation, other than gluten, which occurred at a different time. FBSG includes 32.40% of proteins (Table 2), which underwent modification during the fermentation process, including proteolysis (Fig 1) and changes in tertiary structure due to the drop in pH post lactic acid production. Gluten aggregation is hindered in acidic conditions, and alterations in charges facilitates the formation of new secondary bonds (Bouachra et al., 2017). In addition, these modified protein/peptides may show differences in solubility compared to gluten, which also affects the protein aggregation (Hoehnel et al., 2019) and contributes to the formation of two peaks during the measurement.

3.3 Starch pasting properties

Utilisation of fibre rich ingredients BSG and FBSG in semolina-based pasta formulations influenced starch pasting properties (Table 3).

As a general trend, a reduction in peak and final viscosities was noted upon inclusion of the fibre ingredients. This is consistent with previous findings (Brennan & Samyue, 2004; Collar et al., 2006). Peak viscosity values represent the level of water taken up by starch granules in the presence of heat and shearing. Semolina exhibited the highest peak viscosity (789 ± 33.6 cP). WM had a significantly lower peak viscosity (599 ± 33.3 cP) than semolina due to the increased prevalence of bran particles in WM, which have a higher water-binding capacity and compete with starch for hydration (Rakhesh et al., 2015; Sudha et al., 2007)**.** The addition of BSG and FBSG significantly decreased the peak viscosity. The higher the fibre addition level the lower the peak viscosity (Table 3). Semolina is replaced by low starch, high fibre BSG and FBSG ingredients; therefore the amount of starch present to absorb water and contribute to viscosity is lower in these formulations (Collar et al., 2006; Symons & Brennan, 2004). The peak viscosity for FBSG HF (322 ± 25.4 cP) was significantly lower than BSG HF peak viscosities, putatively due to the increased level of alpha-amylase activity in the FBSG ingredient (Table 2), which hydrolyses the starch polysaccharides and causes a further reduction to viscosity (Ferry et al., 2005).

Similar trends were observed for the final viscosity, which represents the level of starch retrogradation and paste formation. Final viscosity tends to increase with increasing levels of starch (Pongsawatmanit et al., 2002). The higher the inclusion level of BSG or FBSG the lower the amount of retrogradation (BSG (SF: 1403 ± 31.0 cP; HF: 967 ± 20.1 cP) and FBSG (SF 1253 ± 18.6 cP; HF: 540 ± 25.9 cP)). Again, semolina was replaced by low starch, high fibre ingredients which dilutes the starch available to retrograde during analysis. Collar et al., (2006) suggested the increased fibre concentration negatively influences the intermolecular association which occurs in the starch network upon cooling via physical disruption; interference in secondary forces; and sterical hindrance. The higher level of amylase activity in the FBSG ingredient is likely to be influencing the lower final viscosities in FBSG formulations compared to BSG. Alpha-amylases have an anti-retrogradation effect and delay the rate of starch retrogradation (Morgan et al., 1997; Palacios et al., 2004). However, the exact mechanism of how this effect occurs is somewhat unclear (Fu et al., 2014).

Breakdown values indicate the extent of amylose leaching from starch granules during heating and shearing. The breakdown values for semolina (101 ± 7.6 cP), WM (101 ± 2 cP), BSG SF (91.6 ± 4.2 cP) were comparable with no significant differences observed. At the HF addition level for BSG, a significantly lower breakdown value was recorded (41.7 ± 3.5 cP) due to the greater reduction in starch present; therefore a lower level of amylose leaching occurred (Collar et al., 2006). Interestingly, the FBSG ingredient showed a different trend to the BSG ingredient at both inclusion levels. Both breakdown values for FBSG SF (134 ± 6.6 cP) and FBSG HF (104.3 ± 5.9 cP) were significantly higher in comparison to BSG. The increased amylase activity and the resulting starch hydrolysis products produced in the fermented formulations is likely to be a contributing factor to the greater breakdown values observed during heating and shearing.

Starch paste temperatures occur at the onset of the sharp increase in suspension viscosity upon heating. Increases have been noted in paste temperatures with fibre inclusion and were attributed to the restrictive nature of fibre inclusion on swelling and amylose leaching (Collar et al., 2006). However, in this study, paste temperatures for all formulations were aligned with the semolina paste temperature (62.1 °C). This indicates the addition of fibre ingredients BSG and FBSG did not have a major effect on starch pasting temperatures at either addition level.

3.4 Effect of fibre ingredient addition on pasta structure

Analysis of pasta ultrastructure was performed on cooked pasta, which are represented in Figure 3.

Semolina pasta (Fig 3.A) contains gelatinised starch granules along with a well-integrated protein matrix. This is consistent with previous reports (Madhumitha & Prabhasankar, 2011; Tudorică et al., 2002). WM pasta (Fig 3.B) has exposed starch granules and lacks the prevalence of string-like gluten structures, which is also reflected in the weak gluten network highlighted during GlutoPeak analysis. The introduction of bran and germ particles from WM flour caused a disruption to gluten network formation, thus effecting it's continuity (Manthey & Schorno, 2002; Noort et al., 2010).

BSG SF (Fig 3.C) and FBSG SF (Fig 3.D) pasta showed the gluten string-like structures similar to those in semolina pasta. This also coincides with the GlutoPeak analysis in these pasta recipes. However, a different trend was observed in relation to the starch granules. The starch granules appear to have a layer surrounding them, creating a gel-like structure. This gel like layer is amplified in micrographs for BSG HF (Fig 3.E) and FBSG HF (Fig 3.F) due to the increased addition level. BSG contains arabinoxylans (Lynch et al., 2016), which have the unique capability to crosslink and form a gel-like structure when sufficient concentration are present (Courtin & Delcour, 2002; Izydorczyk et al., 1990). The gel-like layer/aggregates observed in BSG and FBSG ultrastructure could be due to interactions between arabinoxylan chains. BSG HF and FBSG HF also lack the distinct gluten structures putatively due to the presence of the arabinoxylans, which negatively affect gluten formation through a physical effect (increasing viscosity and depleting protein interactions) and a chemical mediated effect (interactions between ferulic acids) (Wang et al., 2004). The similarity in SEM micrographs for BSG and FBSG indicates fermentation of BSG did not have a major impact on pasta ultrastructure.

3.5 Impact of fibre ingredients on pasta properties

The effect of fibre fortification on semolina pasta using BSG and FBSG was investigated by evaluating pasta characteristics, such as tensile strength, firmness, stickiness, optimal cooking time and cooking loss (Table 3).

3.5.1 Tensile strength

Tensile strength for semolina pasta was 0.29 ± 0.03 N, which was comparable to previous reports (Hoehnel et al., 2020; Tudorică et al., 2002). WM pasta tensile strength was immeasurable. The WM pasta strands broke whilst attempting to conduct the measurement, highlighting the weak structure of the pasta. This was due to the physical disruption of the large bran and germ particles within the gluten network, which had a negative effect on the continuity of the gluten network and is reflected in SEM images.

The tensile strength of BSG SF pasta (0.27 ± 0.03 N) was not significantly different to the semolina control. A reduction in tensile strength was observed for FBSG SF (0.24 ± 0.04 N), BSG HF (0.16 ± 0.04 N) and FBSG HF (0.15 ± 0.03 N). Tudorică et al., (2002) and Brennan et al., (2004) also found a reduction in tensile strength with addition of inulin and guar gum. The lower tensile strengths in these formulations are potentially due to the presence of the arabinoxylans in the BSG and FBSG ingredients. Arabinoxylans hinder gluten properties negatively, making it less extensible (Wang et al., 2004), hence the negative effects observed in the elasticity of the pasta. The relatively comparable tensile strength recorded for BSG and FBSG pasta indicates fermentation did not influence the elastic properties of the pasta.

3.5.2 Firmness

Torque values from GlutoPeak analysis correlated positively with firmness values for the final pasta ($r=0.871$, $p \leq 0.03$), suggesting gluten network strength influenced the firmness of the final pasta (Table 3).

Semolina pasta had a firmness after cooking value of 2.17 ± 0.37 N, while WM pasta had a significantly lower firmness value (1.47 ± 0.25 N). This aligns with previous reports (Manthey & Schorno, 2002; Padalino et al., 2015). The lower firmness value is likely to be linked with the weaker gluten network formed (GlutoPeak), which allows for a more open, porous structure (SEM) and contributes to the reduction in pasta firmness.

The firmness after cooking of BSG SF (2.27 ± 0.40 N) and FBSG SF (2.54 ± 0.38 N) pasta were marginally higher than the semolina control. This is likely due to the stronger gluten network formed (GlutoPeak) and the increase in protein content with inclusion of high protein ingredients BSG and FBSG. Enhanced protein contents have previously been linked with increased pasta firmness (Manthey & Schorno, 2002;Sissons et al., 2005). BSG HF pasta had a

significantly higher firmness value (2.62 ± 0.65 N) than the semolina control. Again, the stronger gluten network (GlutoPeak) is likely to be influencing this, as well as the further increase in protein concentration with higher levels of BSG. The incorporation of FBSG at the high fibre level decreased the pasta firmness (1.85 ± 0.16 N) compared to BSG, indicating the fermentation of BSG reduced pasta firmness. This could be due to the variations observed in gluten aggregation properties (GlutoPeak) which negatively impacted the firmness of the pasta.

3.5.3 Stickiness Pasta stickiness has been associated with starch pasting properties (Sozer et al., 2007). Furthermore, a strong positive correlation was found with breakdown values from RVA analysis and pasta stickiness ($r=0.9$, $p \leq 0.02$), indicating the level of amylose leaching during cooking influences the stickiness of the final pasta. Additionally, GlutoPeak torque values and stickiness in pasta correlated positively ($r=0.825$, $p \leq 0.05$), suggesting gluten network strength also affects the stickiness of the final pasta (Table 3).

Stickiness of semolina pasta (4.79 ± 0.4 N) and WM pasta (5.23 ± 0.7 N) were not significantly different. Similar stickiness values were obtained for BSG SF (4.26 ± 0.99 N) and FBSG SF (5.04 ± 0.73 N) pasta. An increase in BSG addition showed a significantly reduced pasta stickiness (3.46 ± 0.59 N) putatively due to the lower amount of starch available to gelatinise, the stronger gluten network and the lower level of amylose leaching. The stickiness value for FBSG HF pasta (4.64 ± 0.78 N) was significantly higher than the stickiness of BSG HF pasta. This result coincides with the enhanced breakdown values observed in RVA trials. Chamberlain et al., (1981), found an increase in crumb stickiness in bread with increased alpha-amylase activity and production of high molecular weight dextrins. The higher amylase activity in FBSG may enhance the production of starch degradation products such as dextrins which may increase the stickiness of the surface of the pasta. Additionally, the alterations in protein network formation (GlutoPeak) with FBSG inclusion could negatively influence pasta structure and allow for a greater amount of amylose to leach onto the pasta surface compared to BSG HF pasta. However, the stickiness of the FBSG HF pasta was not significantly different to the semolina control.

3.5.4 Optimal Cooking Time

Changes were observed in optimal cooking time (OCT) with the inclusion of fibre ingredients. A strong positive correlation was noted in OCT and torque values ($r=0.9$, $p \leq 0.02$), as well as OCT and PMT values ($r=0.96$, $p \leq 0.03$) from GlutoPeak analysis. This indicates the strength and speed of gluten formation influences the optimal cooking time of the pasta.

Semolina pasta had an OCT of 5.5 $\pm$ 0 mins. A shorter OCT was noted in WM pasta (4 $\pm$ 0 mins). These results are in agreement with previous findings (Manthey & Schorno, 2002; Padalino et al., 2015; Vignola et al., 2018). The reduction in OCT may be attributed to the disruptive nature of the bran and germ particles in WM flour to the protein network. This provides a clear pathway for water to enter the spaghetti core gelatinise the starch and reduce OCT (Manthey & Schorno, 2002).

Inclusion of BSG and FBSG ingredients increased OCT in comparison to the semolina control. BSG SF and FBSG SF had an OCT of 6 $\pm$ 0mins and 6.5 $\pm$ 0 mins, respectively. A further increase in OCT was noted with inclusion of higher levels of BSG and FBSG ingredients, with both BSG HF and FBSG HF pasta having an OCT of 7 $\pm$ 0 mins. This most likely occurred due to the stronger gluten networks formed in these pasta formulations. Conflicting results have been noted in literature with both increases and decreases in OCT found with fibre addition. Variations in OCT have been attributed to pasta structure and gluten network formation (Aravind et al., 2012, 2013; Chillo et al., 2011; Foschia et al., 2014). The inconsistent results suggest OCT may be reliant on fibre type and gluten network formation. The similarity in OCT for BSG and FBSG pasta formulations indicate fermentation of BSG did not influence the cooking quality of the pasta.

3.5.5 Cooking Loss

Cooking loss has been linked with pasta structure and the ability of the protein network to retain amylose (Foschia et al., 2014; Manthey & Schorno, 2002).

No significant differences were observed in cooking loss between semolina (5.44 $\pm$ 0.82%) and WM (5.20 $\pm$ 0.96%) pasta. This is in agreement with Manthey & Schorno, (2002) and Vignola et al., (2018). The addition of BSG or FBSG did not significantly influence the cooking loss, regardless of the inclusion level (BSG SF (4.95 $\pm$ 0.45%), FBSG SF (5.14 $\pm$ 0.17%), BSG HF (4.88 $\pm$ 0.39%), FBSG HF (5.44 $\pm$ 0.68%)). Aravind et al., (2012), also found similar cooking

losses with semolina pasta and semolina pasta substituted with pollard (up to 30 %). Pasta with FBSG showed a marginally higher cooking loss than BSG pasta which may be associated with the slightly higher level of amylose leaching in FBSG formulations, reflected in the higher breakdown values.

3.6 Impact of fibre ingredient on Glycaemic Index

Inclusion of different fibre ingredients, both soluble and insoluble, have previously shown to be capable of reducing the predicted GI of pasta products, with increasing levels of fibre added having a greater effect (Brennan et al., 2004; Brennan & Tudorica, 2008). Values for predicted GI analysis are illustrated in Table 3.

Semolina pasta had a predicted GI value of 55.09 ± 1.41, which is slightly higher than previous reports (Brennan & Tudorica, 2008) but was still within range of the expected GI for pasta (Björck et al., 2000). WM pasta had a significantly lower predicted GI value (38.99 ± 5.30) than semolina, putatively due to the lower level of digestible carbohydrates and higher fibre concentration in the pasta (Table 3), which reduces GI values (Brennan & Tudorica, 2008).

Predicted GI values for BSG SF (46.86 ± 3.86) and FBSG SF (50.50 ± 2.44) did not differ significantly from the semolina control, most likely due to similar level of available carbohydrates in these pasta formulations (Table 3). BSG HF (27.42 ± 0.73) and FBSG HF (18.57 ± 1.52) pasta had significantly lower predicted GI values than the semolina control, which may be due to the dilution effect of digestible starch with increasing levels of fibre added (Table 3). The differences observed in BSG HF and FBSG HF predicted GI values indicate fermentation of BSG had a greater effect in reducing the predicted GI. Further reductions in starch hydrolysis with inclusion of fermented ingredients versus an unfermented ingredient has previously been noted (Cantatore et al., 2019; Lorusso et al., 2017). This may be due to the combined effect of the slightly higher level of resistant starch (1.3% in BSG HF vs 1.6% in FBSG HF pasta) as well as the presence of lactic acid in the fermented ingredient. Östman et al., (2002), investigated the possible mechanisms responsible for the lower availability of starch for amylolysis in bread and concluded the presence of lactic acid during heat treatment promotes interactions between starch and gluten and reduces the bioavailability of starch.

5. Conclusion

The incorporation of spray-dried BSG and FBSG ingredients improved the nutritional properties of semolina pasta in several aspects. In comparison to the semolina control, the addition of BSG and FBSG created a pasta with an improved nutritional profile by achieving a high fibre claim; and further reducing the predicted glycaemic index of the pasta produced. Furthermore, the addition of BSG and FBSG showed a stronger gluten network formation compared to the wholemeal control, resulting in pasta with improved techno-functional properties such as a stronger tensile strength and firmness. Additionally, fermentation of BSG further improved the predicted glycaemic index of HF pasta. This study highlights the excellent potential of upcycling BSG, the main brewing by-product, to produce highly nutritious pasta and potentially further improve pasta nutritional quality using fermented BSG.

6. Declaration of Competing Interest

The authors declare no conflict of interest.

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9. Tables

Table 1: Pasta recipes expressed as percentage-based on flour, “Source of Fibre” (SF) and “High in Fibre” (HF) recipes shown. BSG represents Brewers Spent Grain and FBSG represents Fermented Brewers Spent Grain

	Control Semolina	Control Wholemeal	BSG (SF)	FBSG (SF)	BSG (HF)	FBSG (HF)
Flour	100.00	100.00	97.50	98.00	85.04	87.84
Ingredient	-	-	2.50	2.00	14.96	12.16
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Water	30.00	36.50	30.00	30.00	36.52	36.52

834 **Table 2:** Compositional results of the flour ingredients incorporated in experimental analysis. “WM”, “BSG” and “FBSG” denoting for wholemeal
835 flour, brewers spent grain flour and fermented brewers spent grain flour, respectively.

Component	Semolina	WM	BSG	FBSG	Literature values for BSG
Protein	13.2	11.4	31.4	32.4	14.2 - 31.0
Moisture	11.7	12.0	4.7	5.0	n.m.
Fat	1.3	1.6	10.3	6.53	3.0 - 13.0
Ash	1.0	1.3	3.7	3.7	1.2 - 4.6
Total carbohydrate by difference	72.8	73.7	49.9	52.37	n.m.
Of which dietary fibre	5.0	7.1	42.6	49.4	Total Fibre
					48.22
Of which sugars	1.4	1.2	0.2	2.9	n.m.
Beta-amylase (cu/g)	49.30 ± 0.09^{a}	35.38 ± 0.35^{b}	3.36 ± 0.01^{c}	3.73 ± 0.20^{c}	n.m.
Alpha-amylase (cu/g)	0.18 ± 0.01^{b}	0.12 ± 0.02^{c}	0.12 ± 0.00^{c}	0.24 ± 0.00^{a}	n.m.
Starch Analysis					
Total Starch	62.88 ± 0.37^{a}	55.55 ± 2.65^{b}	2.31 ± 0.05^{c}	3.75 ± 0.06^{c}	1 - 12
Digestible Starch	56.77 ± 0.40^{a}	48.32 ± 3.02^{b}	1.34 ± 0.04^{c}	2.47 ± 0.02^{c}	n.m.
Resistant Starch	6.11 ± 0.01^{b}	7.22 ± 0.37^{a}	0.97 ± 0.01^{c}	1.27 ± 0.04^{c}	n.m.
Values expressed in g/100g. N.m.= not measured. Literature values sourced from Lynch et al., (2016) and Waters et al., (2012).					

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Table 3: Rapid Visco Analyser, GlutoPeak and pasta characterisation results for "source of fibre" (SF) and "high in fibre" (HF) recipes. BSG and FBSG represent brewers spent grain and fermented brewers spent grain, respectively. WM indicates wholemeal control. Values are given as the average ± standard deviation. No significant difference occurred between values in the same row which share the same letter ($p < 0.05$).

	Semolina	WM	BSG SF	FBSG SF	BSG HF	FBSG HF
Rapid Visco Analyser						
Peak visc. (cP)	789 ± 33.6^{a}	599 ± 33.3^{c}	685 ± 12.1^{b}	701 ± 7.0^{b}	431 ± 14.2^{d}	322 ± 25.4^{e}
Breakdown (cP)	101.0 ± 7.6^{b}	101.0 ± 2.0^{b}	91.6 ± 4.2^{b}	134.0 ± 6.6^{a}	41.7 ± 3.5^{c}	104.3 ± 5.9^{b}
Final visc. (cP)	1527 ± 66.5^{a}	$1317 \pm 28.0^{b,c}$	1403 ± 31.0^{b}	1253 ± 18.6^{c}	967 ± 20.1^{d}	540 ± 25.9^{e}
Paste Temp (°C)	$62.1 \pm 5.8^{a,b}$	73.7 ± 6.8^{a}	$60.7 \pm 8.9^{a,b}$	$64.2 \pm 0.5^{a,b}$	$61.8 \pm 5.5^{a,b}$	50.7 ± 0.4^{b}
GlutoPeak						
Peak Max Time (sec)	91.3 ± 0.6^{b}	126.0 ± 7.5^{a}	72.0 ± 2.0^{c}	65.3 ± 6.1^{c}	21.6 ± 0.6^{d}	45.0 ± 3.0^{e}
Torque Maximum (BEM)	45.0 ± 0.0^{c}	27.7 ± 1.2^{d}	52.0 ± 1.0^{b}	52.3 ± 0.6^{b}	67.3 ± 3.1^{a}	50.3 ± 0.6^{b}
Pasta Characterisation						
Total Average Fibre in Cooked pasta (%)	3.08	4.25	3.68	3.51	6.44	6.12
Optimal Cook time (mins)	5.5 ± 0^{e}	4.0 ± 0^{f}	6.0 ± 0^{d}	6.5 ± 0^{c}	7.0 ± 0^{b}	7.0 ± 0^{a}
Cook Loss (%)	5.44 ± 0.82^{a}	5.20 ± 0.96^{a}	4.95 ± 0.45^{a}	5.14 ± 0.17^{a}	4.88 ± 0.39^{a}	5.44 ± 0.68^{a}
Firmness after cooking (N)	2.17 ± 0.37^{bc}	1.47 ± 0.25^{d}	2.27 ± 0.40^{abc}	2.54 ± 0.38^{ab}	2.62 ± 0.65^{a}	1.85 ± 0.16^{cd}
Tensile Strength (N)	0.29 ± 0.03^{a}	-	0.27 ± 0.03^{a}	0.24 ± 0.04^{b}	0.16 ± 0.04^{c}	0.15 ± 0.03^{c}
Stickiness (N)	4.79 ± 0.40^{a}	5.23 ± 0.71^{a}	4.26 ± 0.99^{a}	5.04 ± 0.73^{a}	3.46 ± 0.59^{b}	4.64 ± 0.78^{a}
Predicted Glycaemic Index	55.09 ± 1.41^{a}	38.99 ± 5.30^{bc}	46.86 ± 3.86^{ab}	50.50 ± 2.44^{ab}	27.42 ± 0.73^{cd}	18.57 ± 1.52^{d}
Resistant Starch (DWB g/100)	1.00 ± 0.00^{b}	0.99 ± 0.04^{b}	1.20 ± 0.02^{a}	1.04 ± 0.04^{b}	0.80 ± 0.04^{c}	1.02 ± 0.03^{b}
Digestible starch (DWB g/100)	69.47 ± 0.65^{a}	61.01 ± 0.06^{b}	68.07 ± 3.2^{b}	68.47 ± 0.26^{a}	59.22 ± 1.61^{b}	61.32 ± 0.73^{b}
Total Starch (DWB g/100)	70.47 ± 0.66^{a}	61.99 ± 0.02^{b}	69.27 ± 3.2^{a}	69.50 ± 0.21^{a}	60.02 ± 1.64^{b}	62.34 ± 0.77^{b}

DWB represents Dry weight basis. (–) denotes "not measurable". No significant difference in values was found between values in the same row which share the same letter ($p<0.05$).

10. Figure Captions

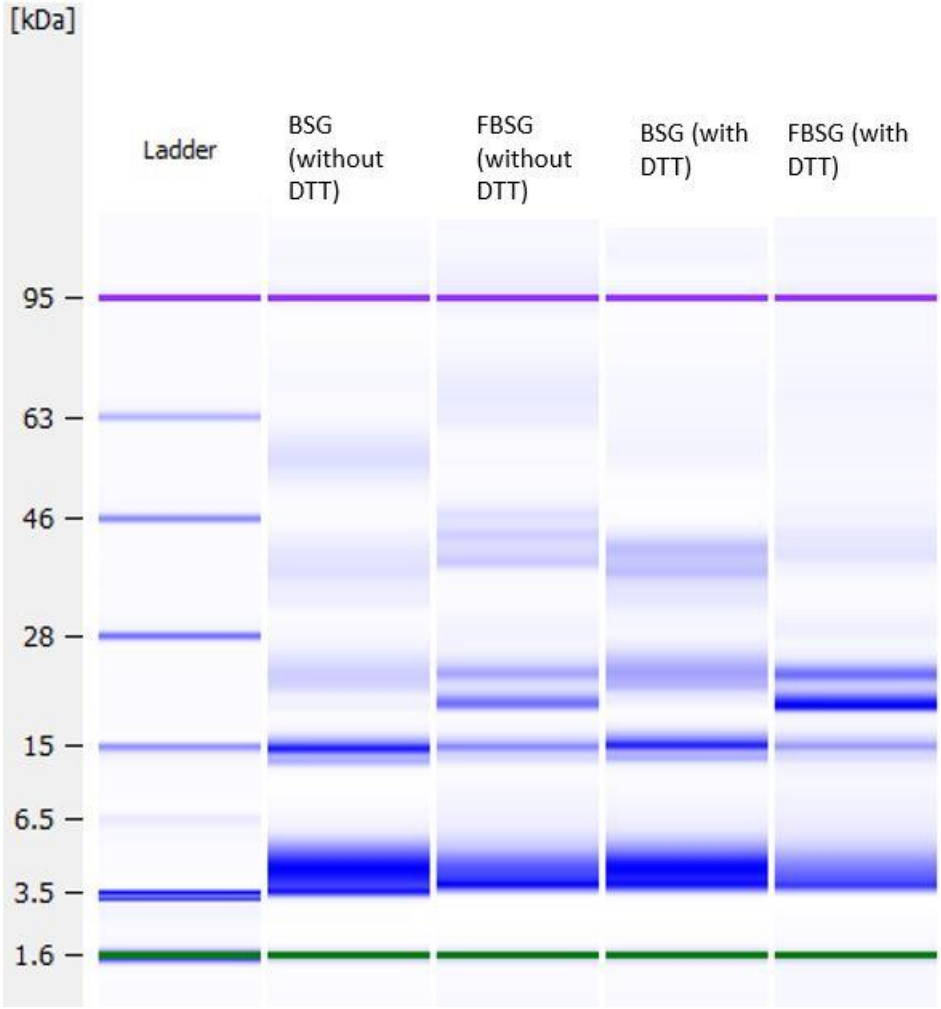
Figure 1. Protein profiles for brewers spent grain (BSG) and fermented brewers spent grain (FBSG) with and without DTT, in the range of 5-80kDa.

Figure 2. Graphical representation of GlutoPeak results from controls and flour mixtures with brewers spent grain (BSG) and fermented brewers spent grain (FBSG) at source of fibre (SF) and high in fibre (HF) addition levels.

Figure 3. Ultrastructure of cooked pasta samples. Image A-F represents semolina (A), wholemeal (B), brewers spent grain “source of fibre” (C), fermented brewers spent grain “source of fibre” (D), brewers spent grain “high in fibre” (E) and fermented brewers spent grain “high in fibre” (F) pasta formulations, respectively.

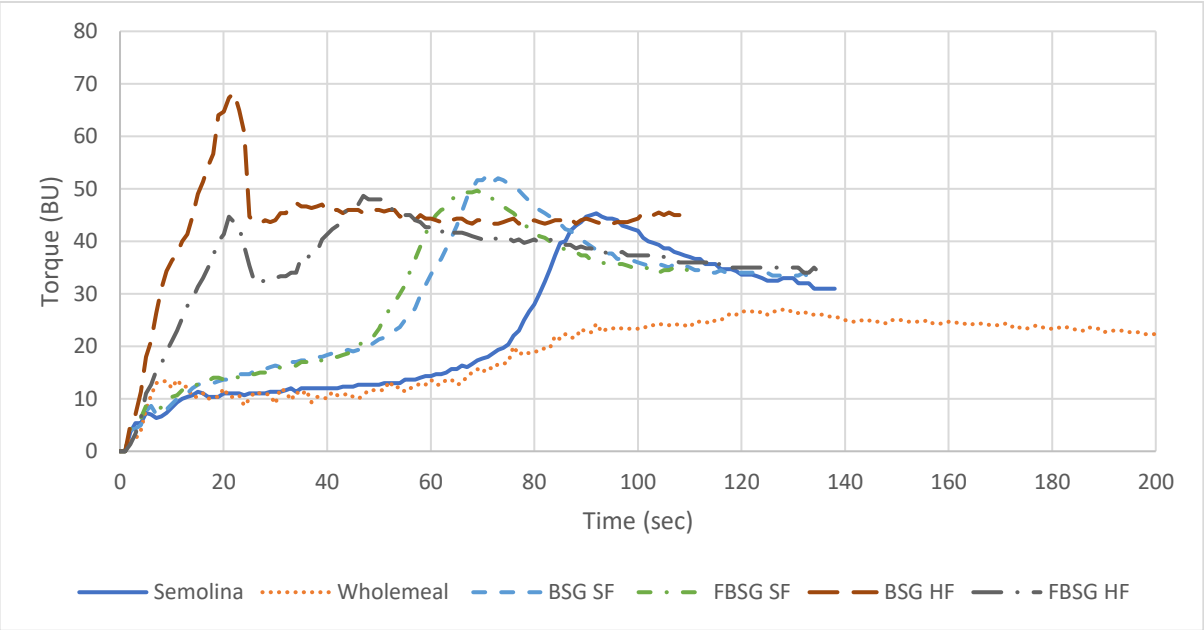
11. Figures

Figure 1



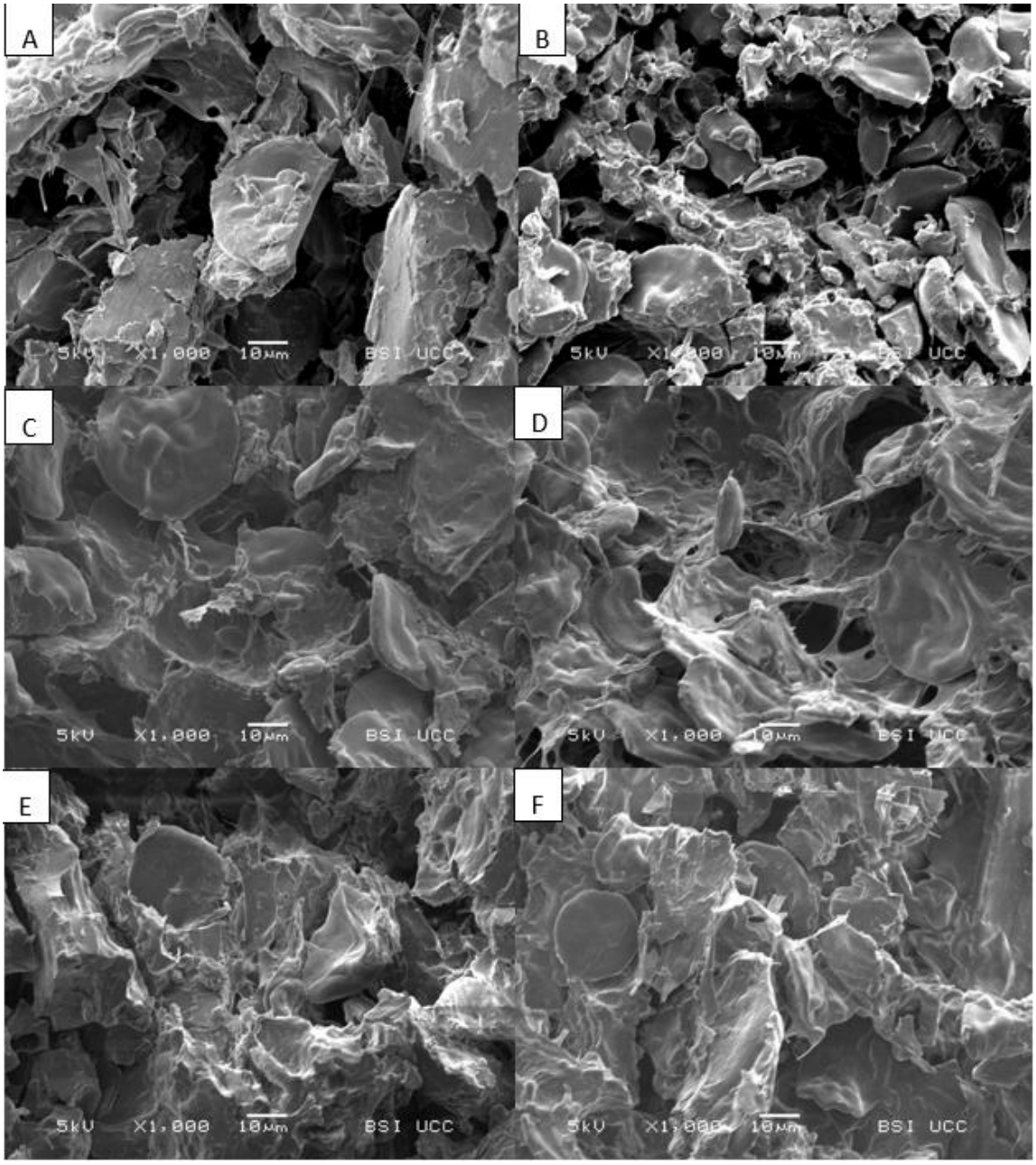
857 **Figure 2**

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860 **Figure 3**



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