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Evaluation of the breadmaking properties of bioprocessed brewer's spent grain

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Abstract

Brewer's spent grain (BSG) is the main by-product of the beer brewing industry. Despite its nutritional quality, and the potential environmental and economic benefits of its use as food ingredient, BSG is currently used as feed or discarded. BSG is a challenging material, due to high water and fibre content, not homogeneous texture and particular flavour. This study explores BSG addition to bread through *in situ* production of dextran by *Weissella confusa* A16, using a "clean-label" strategy. Lactic acid bacteria (LAB) fermentation can improve the sensory properties and bread shelf-life, while dextran improves texture and volume of bread. Sucrose supplemented BSG was fermented by the above strain, added to wheat bread recipe and compared with wheat bread, untreated BSG bread and bread with BSG fermented without sucrose. Results show that BSG was a suitable substrate for the growth of *W. confusa* A16, bringing low acidity, and high dextran production (7.2% on dry weight of fermented BSG). A positive effect of dextran on BSG bread volume and hardness (respectively +13% and -52% than fermented control) was clearly seen, as well as a sensible reduction of the staling rate. Thus, this biotechnology is an effective tool to exploit BSG as bread ingredient, facilitating consumers acceptance.

Riassunto

La trebbia (brewer's spent grain, BSG) è il maggior prodotto di scarto dell'industria della birra. Nonostante il suo uso in alimentazione umana possa avere benefici di tipo nutrizionale, ambientale ed economico, la trebbia è un materiale impegnativo, a causa dell'alto contenuto di fibra e di acqua, della consistenza non omogenea e dell'aroma particolare. Questo studio esamina gli effetti dell'aggiunta di trebbia al pane attraverso la produzione *in situ* di destrano da parte del batterio lattico *Weissella confusa* A16, usando una strategia "clean-label". La fermentazione dei batteri lattici può contribuire a ottimizzare le proprietà sensoriali e la shelf-life del pane, mentre il destrano, prodotto in presenza di saccarosio, migliora la texture e il volume del pane. La trebbia fermentata con aggiunta di saccarosio è stata integrata nella ricetta del pane di frumento, e questo confrontato con pane bianco, pane con trebbia non trattata e pane con trebbia fermentata senza saccarosio. I risultati mostrano che la trebbia è un substrato favorevole alla crescita di *W. confusa* A16, contribuendo a una bassa acidità, e alla produzione sufficiente di destrano (7.2% su peso secco di trebbia fermentata). In queste condizioni, è stato notato un effetto positivo del destrano sul pane con trebbia, riguardo al volume e alla durezza (+13% e -52% rispetto al controllo fermentato), così come si è verificata una sensibile riduzione del tasso di raffermamento. Dunque, questa biotecnologia è uno strumento efficace per aggiungere la trebbia al pane, favorendone l'accettabilità da parte dei consumatori.

1. Introduction

1.1 Brewer's spent grain properties

Brewer's spent grain (BSG) is the main by-product of the beer brewing industry, produced during mashing. Firstly, malted barley is mixed with water and the temperature is raised in order to activate endogenous enzymes, especially carbohydrases, until obtaining desired solutes concentration in the wort; secondly, the wort is filtered through the remaining of the malted barley, that is called BSG (Mussatto et al., 2006; Stewart et al., 2017). This by-product represents 85% of the waste of the brewing process and it is available during the whole year: worldwide, annual regular beer production (based on 2014 data, from 'FAOSTAT', 2020) is 180 million tonnes. Thus, estimating that discarded BSG is approximately 20 kg/hl of beer (Mussatto, 2014), the total amount of BSG produced worldwide should be around 36 million tonnes per year. A similar estimation is reported by Niemi et al. (2012).

Generally, BSG is mainly formed by cell wall residues of pericarp, seed coat and husk of the spent grain with low amounts of starchy endosperm and it may contain residues of hop or cereals different from barley (for instance, wheat) depending on brewing system and beer type (Lynch et al., 2016; Mussatto et al., 2006). Therefore, it is considered a lignocellulosic material, rich in fibres and proteins, anyway the composition can vary significantly due to barley variety, growth conditions, harvest period and production process (Santos et al., 2003). Among fibres, cellulose and hemicellulose represent about 50% on dry weight (d.w.) of BSG composition (Mussatto, 2014), making it a high fibre ingredient. Lignin is considered a dietary fibre as well, when associated with other fibrous polysaccharides (EFSA European Food Safety Authority, 2017), and it is a major component of BSG (10-20% d.w.), followed by proteins (15-30% d.w.) and other components such as lipids, minerals and phenolics (Lynch et al., 2016). Previous studies showed the high nutritional value of BSG's proteins due to the valuable amount of essential aminoacids, in which lysine, generally lacking in cereals, is the most abundant, consisting 14% of total amount of essential aminoacids (Waters et al., 2012). In this matrix, minerals are widely represented with an ash total content of approximately 1-4% d.w., among which the most present is phosphorous (Meneses et al., 2013; Mussatto, 2014). Generally, in plant products, phosphorous is quite nutritionally not available, forming phytate. Phenolic content was studied due to its antioxidant, anti-inflammatory and anti-carcinogenic potential, and ferulic and *p*-coumaric acid were the most abundant, even if found in the bound form (McCarthy et al., 2013).

Despite the significant amount of available BSG, currently it is mainly used as cattle feed (Buffington, 2014). Other uses are energy production, both with direct combustion or for biogas fermentation, use as a substrate for microbes cultivation and for microbes derived enzymes

(Mussatto et al., 2006) or finally it is biorefined to obtain chemical compounds (Mussatto, 2014). Instead, thanks to its low cost, several potential applications focusing on human nutrition could be sought.

The main reasons why adding BSG as food ingredient are the followings. From consumers' point of view, this matrix can have health and environmental benefits: on one side, as described above, previous studies underlined the valuable nutritional properties of BSG for human consumption, from high fibre content, which is claimed to help gastro-intestinal transit, to phenolic beneficial activity (Lynch et al., 2016). On the other side, using it in food is a waste recycling action, and since global concern for food loss and waste has increased, lowering the approximately 1/3 of food lost or wasted worldwide (based on FAO 2011 estimation) is included in the Sustainable Development Goal 12 ('Sustainable Development Goals - FAO', 2020). Lastly, since BSG is a by-product, its cost is still really low (Buffington, 2014), so BSG food profit could be quite substantial.

Despite the above considerations, BSG use as food ingredient presents several challenges which limit its re-introduction in the food chain.

In fact, challenges in managing and handling this material are yet to be solved. Due to the high water content (roughly 75-80%; Mussatto et al., 2006), spoilage can be really fast, if right storage conditions are not observed, leading also to the growth of undesirable microbiota. Bartolomeé et al. (2002) studied different storage methods focusing on the preservation of chemical compounds, in particular pentoses and hydroxycinnamic acids, suggesting that oven drying and freeze drying are preferable to simple freezing, because volumes become smaller and microbial alteration during defrosting can occur, leading to chemical alteration. Actually, BSG microbiota was investigated by Robertson et al. (2010a), pointing out that a fast microbial growth can occur right after mashing. In the same study, authors report that BSG is at risk of rapid colonisation by anaerobic bacteria due to low oxygen availability in the mass. Furthermore, other technological challenges are related to BSG texture and flavour. BSG texture is not homogeneous, being constituted by nearly intact barley husks, so it generally needs further processing such as milling (Ktenioudaki et al., 2015; Steinmacher et al., 2012). Additionally, the high fibre content of this material can lead to detrimental effects in volume and texture of food products. For instance, in breadmaking the fibre polymers interfere with the formation of an optimal gluten structure (Delcour & Hoseney, 2010). Concerning the sensory properties, BSG baked products taste was evaluated, pointing out a more complex and acidulous flavour (malty, buttery hints) than wheat bread (Fărcaş et al., 2015; Ktenioudaki et al., 2015; Waters et al., 2012) and therefore making its acceptability uncertain.

According to our current knowledge, previous works on BSG have been mostly focusing on i) exploring its nutritional and sensory properties, both as such and after fermentation, in bread and

baked snacks (Fărcaş et al., 2015; Ktenioudaki et al., 2013a; Niemi et al., 2012; Waters et al., 2012); ii) studying the rheological properties of BSG-wheat flour doughs and textural properties of bread (Ktenioudaki et al., 2015; Ktenioudaki et al., 2013b; Steinmacher et al., 2012); iii) other applications such as compounds extraction (fibres, proteins and phenolics) and energy or biomass production through biotechnological methods (He et al., 2019; Meneses et al., 2013; Ravindran & Jaiswal, 2016).

1.2 Lactic acid bacteria fermentation

Lactic acid bacteria (LAB) fermentation is a potential solution to overcome BSG challenges.

It is known that sourdough fermentation, a biotechnology dating back to 5,000 years ago, leads to significant improvements of products such as bread and other baked goods, giving better sensory properties and longer shelf-life (Gobbetti & Ganzle, 2013). This type of fermentation is mostly due to LAB, among which a wide taxonomic range is present (e.g. species belonging to the genera *Lactobacillus, Lactococcus, Pediococcus, Weissella* etc), but also yeasts can occur. Organic acids production (i.e. lactic and acetic acid) is the prevalent LAB function in this matrix and the type and ratio of acids depends on the LAB metabolism (Gobbetti & Ganzle, 2013). Organic acids give a mild acidity, that is especially required for bread technology, and a slightly sour taste, improving poor-flavoured ingredients (Cauvain, 2015). Moreover, acidification can lead to nutritional positive effects, such as the release of phenolics from the fibres chains or the breakage of phytate, an antinutritional factor (Çakır et al., 2020; Hole et al., 2012; Reale et al., 2007; Saa et al., 2017).

As previously outlined, BSG is an ingredient with scarce sensory and texture properties, mainly due to the high fibre content. Many studies have already assessed that sourdough fermentation improves sensory quality and storage performance of wheat bread (Katina et al., 2006a; Rizzello et al., 2010) and fibre-rich bread (De Angelis et al., 2007; Mariotti et al., 2014; Pejcz et al., 2017).

Focusing on BSG bread, Waters et al. (2012) compared both sensory and texture properties of breads containing whole-wheat flour and BSG fermented by *Lactobacillus plantarum* strain. They concluded that BSG bread could be a valid alternative to whole-wheat bread, having similar flavour and texture, although a greater hardness and lower sweetness of fermented BSG bread were observed. This negative effect of fermentation was attributed to decreased elasticity of gluten network, probably due to gluten dilution effect and lower pH that modified protein and starch behaviour, other than sugar consumption by LAB for metabolic requirements. In another work, BSG sourdough, fermented by *Lactobacillus brevis*, *Lactobacillus plantarum* and *Saccharomyces*

containing untreated BSG, was compared with BSG bread added with dough conditioner (mixture of enzymes, surfactants and oxidising agents) or xylanase, additives that can improve bread volume (Ktenioudaki et al., 2015). The use of fermented BSG showed a negative impact on bread crumb texture, but a positive effect on sensory parameters, compared to non-fermented BSG bread. In this case, the addition of dough improver was the most effective treatment regarding hardness values.

Therefore, while using LAB fermentation, BSG bread volume and texture are properties that can be improved.

1.3 Dextran biosynthesis in situ *during LAB fermentation*

Several additives can be considered as a solution to improve bread texture and volume, but nowadays consumers tend to prefer natural and "clean" food products. Although, the legislation has not already established a proper definition of "clean-label" product. This type of food is generally produced with "familiar" and healthy ingredients, without "E-numbers" additives, that may be perceived as negative by consumers (Asioli et al., 2017). Thus, "clean-label" strategies are the key to meet industry's needs with consumers' acceptability (Asioli et al., 2017).

In this context, fermentation with *in-situ* production of functional compounds is considerable as a "clean-label" approach, since it does not imply the use of E-codes. Moreover, products gain the beneficial effects of microbial fermentation, as described above. Examples can be found in a variety of different foods, in which microbial fermentation leads to the production of vitamins (Burgess et al., 2004), bacteriocins (Devi et al., 2014), γ-aminobutyric acid (Han et al., 2020), hydrocolloids (Wang et al., 2019) and many others.

In particular, hydrocolloids are water-soluble polymers, often used in bread and other food products to enhance their texture (Norton et al., 2011). In past studies, several authors used bioprocessing and the addition of hydrocolloids in the formulation of bread. These polymers can be introduced in bread as pure ingredient (Zannini et al., 2014) or through fermentation via exopolysaccharides (EPS) producing LAB. LAB can produce two classes of EPS: extracellularly synthesised homopolysaccharides (HoPS), with same unit repeated, and heteropolysaccharides (HePS), formed from different units, in which the first class is the most studied (Tieking & Gänzle, 2005). Among the HoPS, dextran is the most studied for bread applications (Tieking & Gänzle, 2005; Zannini et al., 2014).

Dextran is synthetized from sucrose by the enzyme dextransucrase (1,6-α-D-glucosyltransferase; E.C. 2.4.1.5). This enzyme cleaves sucrose to provide glucose, which is then linked to a main backbone chain through α-(1→6) glucosidic linkage and to the branches with

varying linkages (α-(1→2), α-(1→3), α-(1→4)). The degree and type of branching depends on the origin of dextransucrase, as well as oligosaccharides (e.g. isomalto-oligosaccharides), produced with a secondary reaction called "acceptor reaction" (Whitaker et al., 2003). Isomalto-oligosaccharides are also claimed to have a functional role in food products, as prebiotics and bread dough improvers (Park et al., 2016; Singh et al., 2017).

Among the LAB producing dextran, *Weissella* and *Leuconostoc* spp. are the most studied. *Weissella* and *Leuconostoc* are Gram-positive, catalase-negative, non-endospore forming bacteria with coccoid or rod-shaped morphology, mainly non-motile, heterofermentative with production of D(-)/L(+)lactic acid, CO2 and acetic acid or ethanol (Fusco et al., 2015).

In the last years, several studies have shown the benefits of dextran use in bread making (Lacaze et al., 2007). Dextran biosynthesis was studied in several plant materials, like cereal and legume grains (Galle et al., 2010; Katina et al., 2009; Rizzello et al., 2019). For instance, dextran produced in faba bean and pearl millet sourdough by *Weissella confusa* strains improved significantly bread volume and texture: in the case of faba bean, bread volume increased to levels even comparable to wheat bread (Wang et al., 2019, 2018). As example, the structure of *W. confusa* A16 dextran, detected in pearl millet sourdough, is reported in Figure 1.

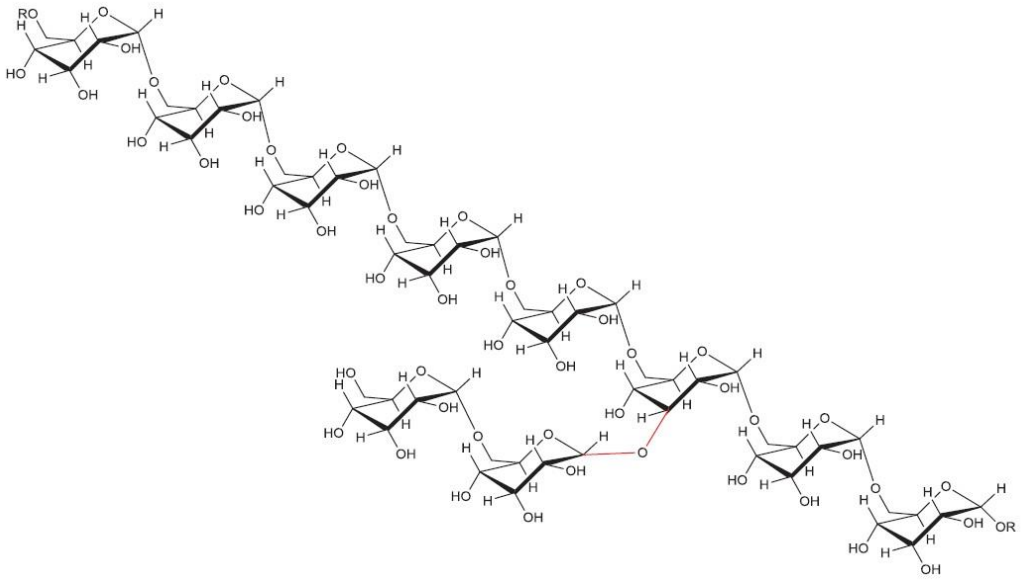


Figure 1 Schematic representation of dextran produced by *W. confusa* A16 (from Wang et al., 2019)

1.4 Aim of the study

The aim of this study is to understand the impact of dextran produced *in situ* during BSG fermentation on wheat bread quality. To the best of our knowledge, no studies are available yet on the incorporation of fermented BSG containing dextran in bread. It is known that bread needs specific inputs, in order to be good, beginning from ingredients. Flour needs to have the right strength and a good quality gluten and starch granules, that give to the dough optimal viscoelasticity (Delcour & Hoseney, 2010). Ingredients needs to bring low acidity and the right oxidation level, that lead to desirable dough development (Cauvain, 2015). Breadmaking has a complex background with numerous factors, and moreover BSG is a challenging ingredient, as explained above.

The hypothesis of the study is that dextran, produced in sufficient amount, can counteract the negative effects brought by the high fibre content of the BSG. *W. confusa* A16 was used to ferment BSG supplemented with sucrose, then bread was baked integrating this BSG in the recipe. A control bread fermented without sucrose and a control bread containing untreated BSG were also prepared and compared to regular wheat bread.

2. Materials and methods

2.1 Materials

BSG was provided from the brewery Dugges Bryggeri, Landvetter, Sweden between March and July 2020 and stored frozen (-20 °C) in the dark until wet milling.

Before use, BSG was subjected to wet milling (Microcut MC15 supported with K04 blade, Stephan Machinery GmbH, Germany). Milling was performed two times in order to have finer texture of BSG paste. Milled BSG (78.1% moisture, standard deviation (s.d.) 0.3, AACC method 44-15.02, 2000; fat 9.9% d.w.; protein 23.4% d.w.; total dietary fibre 47.9% d.w.) was stored frozen (-20 °C) in plastic bags until use in bread or analysis. Frozen BSG was defrosted overnight in a refrigerated cell (4 °C) prior usage.

Ingredients used for breadmaking were BSG (as previously described), wheat flour (moisture 10.2%; Halvgrovt Vetemjol, Helsinki Mylly Oy, Finland), fat (Juokseva rypsiölyivalmiste, Bunge Finland Oy, Finland), fresh yeast (Lahti, Finland), sucrose (Dansukker, Finland) and salt.

2.2 Growth conditions of LAB strain

2.2.1 BSG fermentation

W. confusa A16 (University of Helsinki, Department of Food and Nutrition, own collection) was used as starter for BSG fermentation due to its high dextran production in cereal matrix (Wang et al., 2019, 2020). The strain was maintained at -80 °C in MRS broth supplemented with glycerol (20%, Sigma-Aldrich). For fermented BSG preparation, the strain was routinely cultivated in MRS broth (LABM, UK) at 30° overnight before inoculation. Microbial cells were centrifuged at 12,000 g for 15 minutes, then washed with sodium phosphate saline buffer (PBS, 8.2 g NaCl, 1.7 g K2HPO4·3H2O, 0.2 g KH2PO4 in 1 L Milli-Q water, pH 7.4) and resuspended in a small aliquot (ca. 500 µL) of Milli-Q water, targeting an initial cell density of 10^6 colony forming units (cfu)/g of BSG .

For dextran production (EPS positive BSG, EPS+B), untreated BSG was added with 10% on wet weight (w.w.) of sucrose on EPS+B total weight (11.1% sucrose on wet weight of BSG) and the LAB inoculum described above. For fermented control samples (EPS negative BSG, EPS-B), the LAB inoculum was added without adding sucrose to BSG. BSG was fermented at 25°C for 24 h.

2.2.2 Determination of microbial cell counts

Microbial cell counts were determined at time 0 (after inoculum) and after 24 h fermentation by serial dilutions in sterile saline solution (0.9% weight of NaCl on volume of water). Lactic acid bacteria (LAB) were cultivated on MRS agar (LABM, UK) with pour-plating method and incubated at 30 °C for 48 h in microaerophilic conditions. Total mesophilic counts (TMC) were determined on PCA (Plate Count Agar; LABM) with spread-plating method and incubated at 30 °C for 48 h. *Enterobacteriaceae* were determined on VRGBA (Violet Red Bile Glucose Agar; LABM) with pour-plating method and incubated at 37 °C for 48 h. *Bacillus cereus* was determined on special media modified according to manufacturer instructions (PEMBA with addition of Polymyxin B and egg yolk emulsion; LABM) with spread-plating method and incubated at 30 °C for 48 h. Yeasts and moulds were determined on a substrate prepared with malt extract (30 g/L), soy peptone (5 g/L) and agar n.2 (15 g/L) with the addition of antibiotic (chloramphenicol 0.01%) to avoid the growth of bacteria (LABM). All counts were done in duplicate.

2.3 Determination of pH, total titratable acidity and organic acids

The pH and total titratable acidity (TTA) values of fermented BSG samples were measured as follows: BSG sample (25-30 g) was crushed in a mortar for 3 minutes to rupture its structure, then 10 g of sample were diluted in Milli-Q water and homogenized in a mixer at maximum speed for 1.5 minutes (85 mL final volume of water), then 5 mL of acetone were added. Measurements were done with an automatic titrator (Easy Plus^{TM} Mettler Toledo, USA): TTA was determined as the amount of 0.1 M NaOH required to adjust pH to 8.5.

For organic acid determination, high-performance liquid chromatography (HPLC) method was used. Fermented BSG samples were stored at -20 °C, then freeze-dried and 2 g of sample were mixed with 2 mL of Milli-Q water, vortexed for few minutes, and centrifuged at 10,000 g for 10 min. Supernatant was syringe filtered through 0.45 μm filters (Pall, United states) and filtrate was injected for analysis. The analysis was performed on a Hi-Plex H column (Agilent, CA, USA; 300 × 6.5 mm), with a Hi-Plex H guard column (Agilent, CA, USA; 50 × 7.7 mm). The HPLC system was equipped with a Waters 515 pump, autosampler, ultraviolet (UV) detector (Waters 717), and refractive index detector (HP 1047A, HP, USA). The mobile phase was 10 mM H2SO4 and the flow

rate was set at 0.5 mL/min with the column temperature maintained at 40 °C. Lactic acid (Sigma-Aldrich) and acetic acid (Merck) were used as standards for quantification.

2.4 Dextran analysis

Fermented BSG samples stored at -20 °C were freeze-dried and powdered. Dextran was extracted with an enzyme-assisted method as previously described (Katina et al., 2009). The amount of dextran was determined with high performance anion exchange chromatography with pulse amperometric detection (HPAEC-PAD). The HPAEC-PAD system contains an analytical CarboPac PA-1 column (250×4 mm, i. d, Dionex, Sunnyvale, CA, USA), a Waters 2465 pulsed amperometric detector (Waters, Milford, MA, USA), a Waters 2707 autosampler, and three Waters 515 HPLC pumps. The eluents used were Milli-Q water and 200 mM NaOH as the mobile phase at a flow rate of 1.0 mL/min. Glucose (Merck, Germany) was used as the external standard and 2-deoxy-galactose as the internal standard for quantification.

2.5 Baking procedure

Bread recipes for wheat bread (WB), untreated BSG bread (BB), EPS negative BSG bread (EPS-BB) and EPS positive BSG bread (EPS+BB) are reported in Table 1: in order to have same amount of water in each dough (60% on flour + BSG dry matter), BSG initial moisture (78%) was taken into account.

For bread preparation, all ingredients were mixed in a DIOSNA mixer bowl (Dierks & Söhne GmbH, Germany) for 3 minutes at low speed and 4 minutes at fast speed, adjusting water temperature in order to reach a final dough temperature of 26 ± 1 °C. Dough was rested for 15 minutes in fermentation cabinet (Lillnord, Odder, Denmark) at 35 °C and 75% relative humidity, then it was divided in 250 g pieces, molded manually and proofed in pans for 45 minutes (at 35 °C and 75% relative humidity). Baking was performed in a rotating convection oven (Sveba Dahlen, Fristad, Sweden) at 200 °C for 15 minutes with 15 seconds steaming at the beginning, afterwards breads were depanned and cooled at room temperature for 1h before storage in plastic bags.

Two independent baking trials were done in two different days and six loaves were prepared for each bread type.

2.6 Volume and texture analysis of breads

The loaf volume was determined after 1 day of storage at room temperature by a VolScan Profiler (Stable Micro Systems, UK) and specific volume was calculated by dividing the loaf volume (mL) by the weight (g).

Texture Profile Analysis (TPA) was performed with a texture analyser (TA-XT2i, Stable Micro Systems, UK) using a 25 mm diameter aluminium probe on days 1 and 4: bread parameters (hardness, springiness, cohesiveness, chewiness, resilience; see Table 2 for definitions) during storage were determined with 40% compression according to the AACC Method 74-09 (1998) as described elsewhere (Katina et al., 2006b). Bread samples were prepared by cutting 25 mm x 25 mm x 25 mm cubes of crumb (nine samples originating from three loaves).

Baking loss was calculated as the decrease of weight between dough and loaf (% bake loss = (dough weight – bread weight) * 100/dough weight). Staling rate was calculated as hardness difference in 4 days of storage (staling rate = hardness (day 4 – day 1)/days of storage).

2.7 Determination of pH and TTA in bread crumb

The pH and TTA values of bread crumb samples were measured by homogenizing 10 g of sample in 95 mL Milli-Q water for 1 minute using a Bamix blender, then 5 mL of acetone were added. Measurements were done as described above.

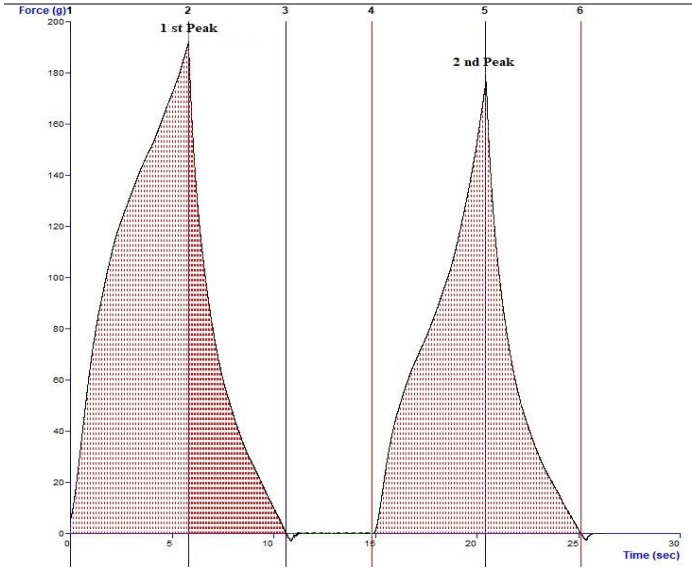
2.8 Statistical analysis

Analysis of statistically significant difference was performed with one-way ANOVA using IBM SPSS Statistics 25 (IBM SPSS Inc., USA) on all data with Tukey's test (significance level $P < 5\%$).

Table 1 Recipes for fermented BSG and BSG bread doughs (f.w. = on flour weight)

	Wheat bread (WB)		*Untreated BSG bread (BB)*		*EPS negative BSG bread (EPS-BB)*		*EPS positive BSG bread (EPS+BB)*	
	g	% f.w.	g	% f.w.	g	% f.w.	g	% f.w.
BSG			523.3	66.7	523.3	66.7	526.0	72.5
Sucrose							58.4	
Fermented BSG					523.3	66.7	584.4	80.5
Wheat flour	900	100.0	784.9	100.0	784.9	100.0	725.9	100.0
Water	540	60.0	131.9	16.8	131.9	16.8	129.8	16.54
Salt	13.5	1.5	13.5	1.7	13.5	1.7	13.5	1.7
Sugar	18.0	2.0	18.0	2.3	18.0	2.3	18.0	2.3
Yeast	45.0	5.0	45.0	5.7	45.0	5.7	45.0	5.7
Fat	54.0	6.0	54.0	6.9	54.0	6.9	54.0	6.9
Total	1570.5	175.0	1570.5	200.1	1570.5	200.1	1570.5	213.7

Table 2 Texture Profile Analysis of BSG bread as calculated by Exponent TA-xT2i program (definitions adapted from Bourne, 2002)

Graph	*Parameter*	*Definition*
	Hardness	Height 1st peak (g) Maximum force for 40% compression
	Springiness	Length 2nd peak (sec) / Length 1st peak (sec) Tolerance to deformation after 1st compression
	Cohesiveness	Area 2nd peak (g*sec) / Area 1st peak (g*sec) Ability to keep structural integrity
	Chewiness	Hardness x Cohesiveness x Springiness Energy required to masticate a solid food product
	Resilience	Area decompression (2:3) 1st peak / Area compression (1:2) 1st peak Ability to recover after compression

3. Results

3.1 Analysis of fermented BSG

3.1.1 Microbial cell counts

Growth of presumptive LAB and total mesophilic counts (TMC) followed the same pattern in fermented BSG with sucrose (EPS+B) and without sucrose (EPS-B), as reported in Table 3, with an increase of ca. 3 Log cfu/g of fermented BSG in 24 h of incubation at 25 °C.

Enterobacteriaceae were detected (ca. 4.0 Log cfu/g) only in EPS+B after 24 h fermentation, instead they were not detected ($<10^2$ cfu/g) in other samples. *Bacillus cereus* and yeasts and moulds were not detected ($<10^3$ cfu/g) in any of the samples.

Table 3 Number of LAB and TMC (Log cfu/g) (n=2-3)

	LAB		*TMC*	
	T0	T24	T0	T24
EPS-B	6.5 ± 0.1^a	9.8 ± 0.2^b	6.6 ± 0.0^a	10.0 ± 0.3^b
EPS+B	6.4 ± 0.0^a	9.4 ± 0.1^b	6.7 ± 0.0^a	9.8 ± 0.1^b

Different letters in the same group (parameter) indicate statistical significance ($p < 0.05$).

3.1.2 Organic acids quantification, pH and TTA

Results of organic acid quantification, pH and TTA after 24 h of fermentation at 25 °C are shown in Table 4. Before fermentation, lactic and acetic acids were not detected, and acidity was ca. 2.0 mL NaOH for both samples. After 24 h of fermentation, the acidity of fermented BSG was slightly higher for EPS positive samples, ranging from 5.4 mL NaOH of EPS negative samples to 6.5 mL NaOH of EPS+B. Similarly, pH values differ of ca. 0.4 between the fermented samples. These results were supported by lactic acid values, because EPS-B and EPS+B amounts are statistically different and they range from ca. 270 mg/100g of fermented BSG (1.2 % on BSG dry weight) to ca. 400 mg/100g of fermented BSG (1.8% on BSG dry weight). No statistical difference in acetic acid values (ca. 0.4% on BSG dry weight) was found between EPS-B and EPS+B.

Table 4 Amount of organic acids (mg/100g of fermented BSG) and acidity (pH and TTA) of BSG after 24h of fermentation

	EPS-B	*EPS+B*
pH	4.8 ± 0.0[a]	4.4 ± 0.0[b]
TTA (mL)	5.4 ± 0.4[a]	6.5 ± 0.1[b]
Lactic acid (mg/100g)	272.0 ± 47.7[a]	398.9 ± 7.8[b]
Acetic acid (mg/100g)	74.0 ± 39.3[a]	81.7 ± 9.4[a]

Different letters in the same group (parameter) indicate statistical significance ($p < 0.05$).

3.1.3 Dextran quantification

The HPAEC-PAD analysis gave results as follows. The EPS-B samples contained ca. 0.2% of dextran on dry weight of fermented BSG, both before and after fermentation. The EPS+B samples contained 7.2% (s.d. 0.3) of dextran on dry weight of fermented BSG.

3.2 Analysis of bread

3.2.1 Volume, TPA and acidity

Breads with 40% w.w. substitution of BSG, as recipes in Table 1, gave results as shown in Table 5. Figure 2 shows the visual appearance of breads.

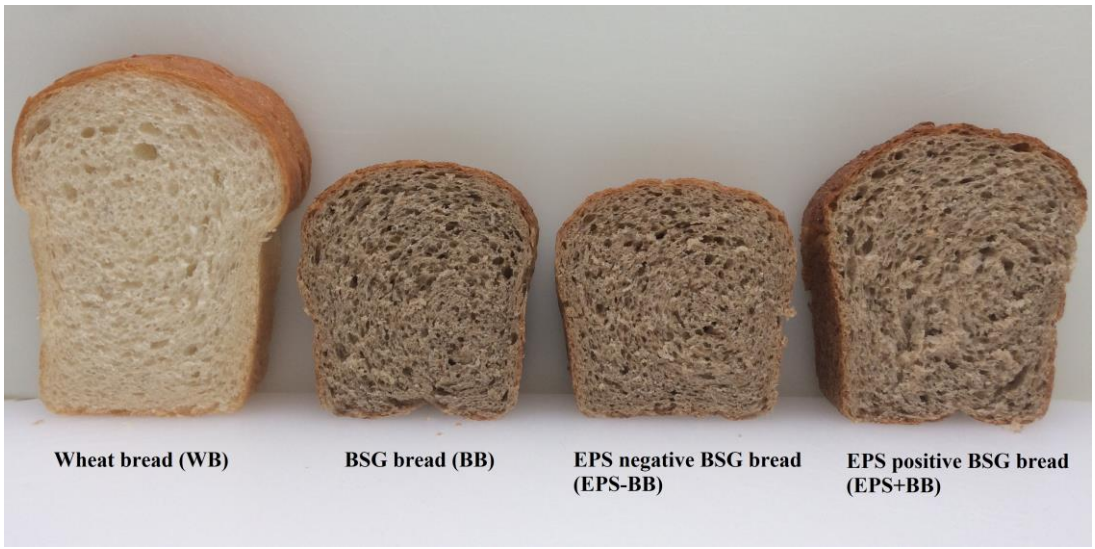


Figure 2 Visual comparison of WB, BB, EPS-BB and EPS+BB

The baking loss was the highest in wheat bread (13.7%), while it ranged from ca. 10 to 11% for the other breads. Overall, specific volume and hardness parameters values are statistically different for each type of bread, indicating a good grouping of samples. The specific volume was the highest in WB and decreased in the BSG containing breads, ranging from 3.4 mL/g (EPS+BB) to 2.9 mL/g (EPS-BB). Hardness at first day was the lowest for WB, while it increased in other types of bread, ranging from ca. 280 g (EPS+BB) to ca. 590 g (EPS-BB). The same pattern was found for hardness at day 4, among which WB value is the lowest (ca. 287 g) and EPS-BB value is the highest (ca. 950 g). Therefore, staling rate, defined as hardness increase (g) per day (d), was different for each bread, ranging from 34.3 g/d of WB to 89.8 g/d of EPS-BB. For springiness, cohesiveness, chewiness and resilience, EPS+BB is statistically different from WB and BB, but not from EPS-BB regarding cohesiveness. Springiness ranges from 0.97 (WB) to 0.90 (EPS+BB); cohesiveness ranges from 0.73 (WB) to 0.65 (EPS-BB and EPS+BB); chewiness ranges from 105.9 (WB) to 350.5 (EPS-BB); resilience ranges from 0.38 (WB) to 0.30 (EPS+BB).

Summarising, EPS+BB was statistically different both from BB and from EPS-BB, concerning all values except of baking loss.

Table 5 Technological parameters of BSG breads

	WB	*BB*	*EPS-BB*	*EPS+BB*
Baking loss (%)	13.7 ± 0.5^{a}	10.4 ± 0.6^{b}	10.3 ± 0.6^{b}	11.1 ± 0.6^{b}
Specific volume (mL/g)	5.1 ± 0.1^{a}	3.0 ± 0.0^{c}	2.9 ± 0.0^{d}	3.4 ± 0.1^{b}
Hardness (g/day 1)	149.5 ± 17.3^{a}	455.3 ± 94.9^{c}	592.3 ± 87.5^{d}	281.8 ± 31.1^{b}
Hardness (g/day 4)	286.6 ± 43.1^{a}	778.9 ± 116.2^{c}	951.4 ± 127.0^{d}	497.3 ± 59.9^{b}
Staling rate (g/d)	34.3^{a}	80.9^{c}	89.8^{d}	53.9^{b}
Springiness (day 1)	0.97 ± 0.02^{a}	0.93 ± 0.01^{b}	0.92 ± 0.02^{b}	0.90 ± 0.02^{c}
Cohesiveness (day 1)	0.73 ± 0.02^{a}	0.67 ± 0.02^{b}	0.65 ± 0.01^{c}	0.65 ± 0.01^{c}
Chewiness (day 1)	105.9 ± 10.7^{a}	284.1 ± 53.7^{c}	350.4 ± 45.8^{d}	163.8 ± 15.8^{b}
Resilience (day 1)	0.38 ± 0.02^{a}	0.33 ± 0.01^{b}	0.31 ± 0.01^{c}	0.30 ± 0.01^{d}

Different letters in the same group (parameter) indicate statistical significance ($p < 0.05$).

Regarding acidity, breads with 40% w.w. substitution of BSG, as recipes in Table 1, gave results as shown in Table 6. Fermented BSG containing breads have lower pH (ca. 5) than WB and BB (ca. 6). Acidity of breads is statistically different between fermented and untreated samples, ranging from ca. 3 mL of NaOH (WB and BB) to ca. 5 mL of NaOH (EPS-BB and EPS+BB).

Table 6 Acidity (pH and TTA) of bread crumb (n=3)

	WB	*BB*	*EPS-BB*	*EPS+BB*
pH	5.9 ± 0.1[a]	5.8 ± 0.1[a]	5.3 ± 0.1[b]	5.1 ± 0.1[b]
TTA (mL)	3.2 ± 0.1[a]	3.4 ± 0.1[a]	5.0 ± 0.4[b]	5.5 ± 0.1[b]

Different letters in the same group (parameter) indicate statistical significance ($p < 0.05$).

4. Discussion

BSG is a challenging material for food use, due to its poor sensory and texture properties and its fast perishability. Thus, its addition in food products needs to be explored from the technological point of view, to implement its use as food ingredient.

In this study, BSG was used as ingredient in bread making, as such or after fermentation. BSG was used wet, exploiting the original water content. Although this might speed the spoilage if not stored properly, this solution requires no additional process, saving energy and time during the production phase.

The maximum percentage of BSG was determined with preliminary tests using a Brabender Farinograph (50 g mixing bowl at 30 °C; data not shown). The substitution of native BSG with wheat flour was fixed at 40% w.w. (14% d.w.), since it allowed the formation of dough; in fact, the incorporated amount was in accordance with previous rheological studies (Ktenioudaki et al., 2013b).

BSG microbiological quality was evaluated in previous studies, showing that different microbial groups can colonise this substrate relatively fast, such as yeast and moulds, aerobic mesophilic and thermophilic bacteria, and microaerophilic and anaerobic bacteria (Robertson et al., 2010a, 2010b). The beer brewing process contributes to a large extent for the narrowing of initial microbial variability of barley grains (Laitila et al., 2007). Yeasts and moulds growth, accordingly, were not detected (<3 Log cfu/g before fermentation) in the spent used here, as well as other types of contaminating agents such as *Enterobacteriaceae* and *B. cereus.* In the present study, results agree with the literature, concluding that native BSG storage was performed correctly and frozen storage with subsequent defrosting at 4 °C did not lead to the growth of potentially harmful or spoilage microbes.

Fermentation with LAB starter producing dextran caused several changes in BSG, both microbiological and chemical. First, *W. confusa* is a species identified in different food environments including cereal sourdough (Fusco et al., 2015; Gobbetti & Ganzle, 2013). This starter was selected since it showed a great capacity to produce dextran and adaptability to several substrates (Wang et al., 2019, 2020). BSG, inoculated with *W. confusa* A16, presented an increased presumptive LAB cell density and brought to a consistent microbial growth (9-10 Log cfu/g) after incubation at 25 °C. Here, *Enterobacteriaceae* were found at a cell density of ca. 4.0 Log cfu/g, which remains within recommended safety limits (International Association of Microbiological Societies, 1986). Previous studies on sourdough fermented by *W. confusa* in similar conditions showed comparable results, in different matrices such as faba bean (Rizzello et al., 2019; Wang et

al., 2018), pearl millet (Wang et al., 2019) and wheat (Katina et al., 2009), confirming that BSG is a suitable substrate for this species.

The acidification properties of fermented BSG were in accordance with previous studies that used the same strain to ferment pearl millet (Wang et al., 2019), but it was lower than in other studies in which faba bean or sorghum were used as substrates (Wang et al., 2018, 2020). Previously, BSG fermentation for incorporation in bread was performed using *Lactobacillus* spp. strains, generally raising the acidity to high levels that are not preferable in the present case, even if lactobacilli are the mainly used LAB in sourdoughs (Ktenioudaki et al., 2015; Waters et al., 2012). *Weissella* spp. metabolism generally produces less acidity because fructose deriving from sucrose addition is not converted to mannitol, thus it does not form acetate (Fusco et al., 2015; Galle et al., 2010), as found also in this study. During fermentation, lactic acid was mostly produced, while acetic acid content was lower; however, both the acids amount is slightly higher than those seen in pearl millet (Wang et al., 2019).

In different studies, it was noticed that sourdough fermented with *W. confusa*, producing dextran and enabling low acidity, was more successful in improving texture and volume of bread than what observed for other more acidifying species, such as *Leuconostoc* spp. (Galle et al., 2010; Tieking & Gänzle, 2005; Wang et al., 2018).

Currently, the species *W. confusa* is not included in the LAB species list approved as GRAS or having QPS status (Koutsoumanis et al., 2020; 'Microorganisms & Microbial-Derived Ingredients Used in Food Partial List - GRAS - U. S. Food & Drug Administration', 2018), although it does not seem to raise matters of concern for healthy humans (Sturino, 2018). Thus, for commercial purposes, each strain safety properties should be tested specifically, before being added as a starter culture. Anyway, in this study, this strain was used because it gave better behaviour in preliminary tests using the Brabender Farinograph (conditions described above; data not shown). Future studies will potentially be able to find a safe EPS producing strain that can have same or better improving results in fermented BSG breads.

The type of dextran produced by *W. confusa* A16 was analysed in previous studies (Wang et al., 2019, 2020). This dextran has a α-(1→6) linked backbone chain of glucopyranosyl residues with 3% of α-(1→3) side linkages (Figure 1) and a molecular weight of 3.3 x 10^6 g/mol. In general, dextran produced by *Weissella* spp. strains presents a low level of branching, with 3-4 % α-(1→3) linkages (Fusco et al., 2015). The structure of dextran is a paramount feature to consider, as it influences the rheological properties of the matrix it is added to (Norton et al., 2011). For instance, in baking applications, dextran is reported to be more effective if molecular weight is relatively high (10^6-10^9 g/mol), giving a softer bread during storage in respect to low molecular weight one (Zhang et al., 2018).

Here, the level of dextran produced in fermented BSG is ca. 1.6% w.w. of the total weight (7.2% d.w.), which is noticeably higher than previously reported in wheat, millet and sorghum sourdough under similar fermentation conditions (Katina et al., 2009, Wang et al., 2019, 2020) and it confirms that BSG provides a suitable environment for dextran production. Theoretically, with 10% sucrose supplementation, only ca. 5% of dextran can be synthesized (Karthikeyan et al., 1996). *W. confusa* was able to form 4.3% of dextran out of the 10% w.w. of sucrose added, that is in accordance with what previously seen in sorghum (Wang et al., 2020). The yield was greater than in wheat sourdough, probably due to the fact that wheat flour normally presents a considerable amount of maltose, that inhibits the formation of dextran and favours the production of oligosaccharides (Katina et al., 2009).

Based on the considerations above, amount of dextran in final bread weight was ca. 0.59% (value calculated according to the bread recipe), which corresponds to 0.92% on flour weight (f.w.). The quantity was comparable with similar studies (Wang et al., 2018, 2020) and it was in the range of addition (0.3-2% f.w.) of hydrocolloids such as hydroxypropyl methylcellulose, necessary to confer improved structural quality to the bread (Armero & Collar, 1997; Zannini et al., 2014). For the European legislation, dextran produced by selected species of LAB in food preparations for bakery products is not a matter of concern for consumers' health, until 5% on final product weight (European Commission, 2001), so this quantity can be considered as safe.

Oligosaccharides analysis and sugar quantification, which are performed to complete microbial metabolic profile during dextran production, are ongoing. It is possible to assume from previous similar studies that some fructose is released from dextran synthesis (Wang et al., 2019; Xu et al., 2017), which could have supported yeast growth during proofing, thus further enhancing bread volume (Galle et al., 2012).

In this study, BSG was used as it was originated from the brewing process, with an initial water content of 78%, and not dried. In fact, in previous studies (Bartolomeé et al., 2002; Meneses et al., 2013), BSG was oven dried at 60 °C after production, most likely inducing an activation of endogenous amylases and leading to the production of maltose, an inhibitor of dextran synthesis (Bertoft et al., 1984; Rodrigues et al., 2005). Nevertheless, dried BSG usage for *in-situ* dextran production needs to be further studied, because drying can prolong BSG shelf-life, easing its handling for industrial purposes.

From the nutritional point of view, this study used a recipe that potentially allows BSG bread to obtain two important claims referred to Regulation (EC) N. 1924/2006 on nutrition and health claims made on foods. Firstly, dietary fibre content is 3.9g/100g of final bread weight (as calculated from BSG dietary fibre content in the recipe), so claiming the product as "source of fibre" is possible. Secondly, about 14% of the energetic value of the product is provided by proteins (BSG presents more than 20% d.w. of proteins), then the label "source of protein" is applicable. These

findings, supported by a complete nutritional evaluation, can lead to a broad exploitation of this ingredient in bread due to its potential appeal in the market, as proposed elsewhere (Mussatto et al., 2006; Lynch et al., 2016).

Breadmaking is a complex technology, and many factors influence bread performance. Previous works (Ktenioudaki et al., 2015; Waters et al., 2012) on breads with similar BSG incorporation (15% substitution on dry weight) are not completely comparable with the present study, because breadmaking was performed in a different way (shorter dough mixing time, lighter loaves, different cooking time and temperature) and the control wheat bread was in one case absent and in the other case much smaller (2 mL/g of specific volume). Thus, other types of bread with similar fibre content (3-4% on final bread weight), baking procedure and wheat bread values are henceforth considered as relatable.

Thanks to dextran supplementation, bread was significantly improved, as also shown in Figure 2. Specific volume increased more than 13% in EPS+BB compared to BB and EPS-BB, while hardness decreased of 38% compared to BB and of 52% compared to EPS-BB. These results bring to the conclusion that dextran was able to recover both fibre and acids detrimental effects, as also shown in previous studies on pearl millet (Wang et al., 2019) and sorghum (Wang et al., 2020) *in situ* EPS-supplemented sourdough bread.

Dextran role in improving bread volume depends on its structural properties. It was shown that the linearity of the polymer, with low number of side chains, can improve loaves volume better than highly branched dextran (Lacaze et al., 2007). Moreover, dextran addition seems to be more effective when a strong gluten network is not present (Yan et al., 2016). The most supported hypothesis is that dextran can help dough cells stability lining up with the gluten matrix, thus improving gas retention during proofing time (Lacaze et al., 2007). Although, a study that compared dextran, xanthan and hydroxypropyl methylcellulose appeared to show limited gas cells expansion in dextran-supplemented dough (Zannini et al., 2014). However, the physical mechanism is still unclear.

Regarding hardness changes and staling, more studies are available. Most likely, dextran increases water absorption of bread dough, giving a softer crumb and a shorter staling rate (Wang et al., 2019; Zannini et al., 2014). It was suggested that dextran could retard bread ageing by partially inhibiting the formation of amylopectin crystallites, involved in the retrogradation process (Zhang et al., 2018). In fact, starch plays a main role in influencing the texture properties of bread crumb (Keetels et al., 1996). Starch granules adhere to the gluten matrix that forms dough cell walls, and swell during hydration and then gelatinization, thus absorbing water from nearby and turning dough foam structure into crumb sponge structure during baking (Delcour & Hoseney, 2010). Dextran can influence this process by limiting water mobility, thanks to its water-holding properties (Lynch et al., 2018). A decreased mobility of water can restrain crumb drying and staling processes, besides

baking loss. In the present study, baking loss was not statistically different between the three types of BSG-supplemented bread, matching ca. 10% on dough weight. This can be due to fibre and protein content of BSG, that are quite higher than in previous similar studies (Wang et al., 2019, 2020), even though water-holding capacity of these polymers in this matrix is yet to be tested. Further research is necessary to understand the actual availability of BSG native water, especially concerning gluten matrix formation.

Springiness and cohesiveness decreased in composite bread, as shown in a similar study (Wang et al., 2019). In fact, gluten-starch matrix is diluted, and elastic properties of the crumb may be compromised, leading to lower recovering ability and structural integrity (Keetels et al., 1996). Noticeably, EPS+BB had the best value only for the chewiness parameter, indicating that dextran supplementation in BSG bread may give a better mouth feel, more relatable to white bread characteristics (Bourne, 2002).

The acidity of breads containing fermented BSG agrees with similar studies in which *W. confusa* was used as starter (Katina et al., 2009; Wang et al., 2019). Additionally, breads acidity is lower than in other sourdough breads studies, thus it is possible to assume that acidic flavour can be mild, thus affecting less bread acceptability (Paterson & Piggott, 2006). Further analyses will be performed to evaluate the sensory properties of these breads.

5. Conclusion

In this study, BSG was used as ingredient in bread making with or without the presence of dextran synthesized *in situ*. The study shows that BSG addition causes deep changes in bread quality, compromising its acceptability. LAB fermentation with dextran production *in situ* partially recovered the negative effects brought by high fibre content, identifying as an effective tool to add spent grain in bread preparations. Moreover, higher nutritional quality of the bread was also achieved due to high fibre and protein content brought by BSG addition.

These findings are important from consumers' point of view, because bread obtained with this technology meets several requests, such as fibre addition, food waste recycling and "clean-label" status. BSG bread acceptability and sensory parameters will be studied to explore the flavour traits of the product and the impact of fermentation and dextran supplementation.

Future studies will focus on the optimization of the spent drying process, which can lead to prolong its durability without compromising dextran producing ability of LAB. Rheological studies on dough supplemented with BSG, as such or fermented, can clarify the process of gluten network formation and the structural role of dextran in this matrix. Further research can investigate the nutritional benefits of the fermentation, concerning the bioavailability of BSG compounds and the microbial metabolites.

This thesis constitutes a first sight into the introduction of dextran in BSG bread.

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