



DIPARTIMENTO DI SCIENZE AGRARIE ALIMENTARI E AMBIENTALI

MASTER DEGREE : FOOD AND BEVERAGE INNOVATION AND MANAGEMENT

EVALUATION OF PORK SAUSAGES QUALITY  
BY THE ADDITION  
OF BREWER'S SPENT GRAINS

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ACCADEMIC YEAR 2018-2019

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# 1 Introduction

## 1.1 What it is brewers' spent grain

Beer is the fifth consumed beverage in the world after tea, soft drinks (carbonates), milk and coffee.

The estimated production in the world in 2002 was over 1.34 billion of hectolitres (Fillaudeau et al., 2006).

The most producer is United States of America with 23 billion litres/year, followed by China with 18 billion per year and Germany 10.5 billion per year (Berto, 2003)

In the beer production spent grain is the main by-product generated around 85% of the total. Starting from the original malt weight around the 31 % approximately 20kg per 100 l of beer produced (Reinold, 1997), in 2002 generated around 1.7 million tonnes of spent grain.

The cereal used for the beer production is barley, for this reason and to animal feed purpose result be the main important cereal after the wheat ( Kendal,1994).

Barley grain is rich in protein and starch and in composed in 3 main parts: the germ (embryo), the endosperm (aleurone and starchy endosperm) and the grain coverings. The part can be also divided in three fractions : innermost layers around the aleurone part, the seed coat and the overlying the seed coat ( pericarp ) that is covered by husk (Fig. 1)( Kunze, 1996).

In the brewing process, the first stages for the barley is the harvested, the cleaning and the selection for different size and after malted separately. After those phases of process the barley is submitted to a period of dormancy of 4-6 weeks. After it is malted under controlled germinated process that increase the concentration of the of enzyme on the grain.

Malting involve three part:

1) maceration;

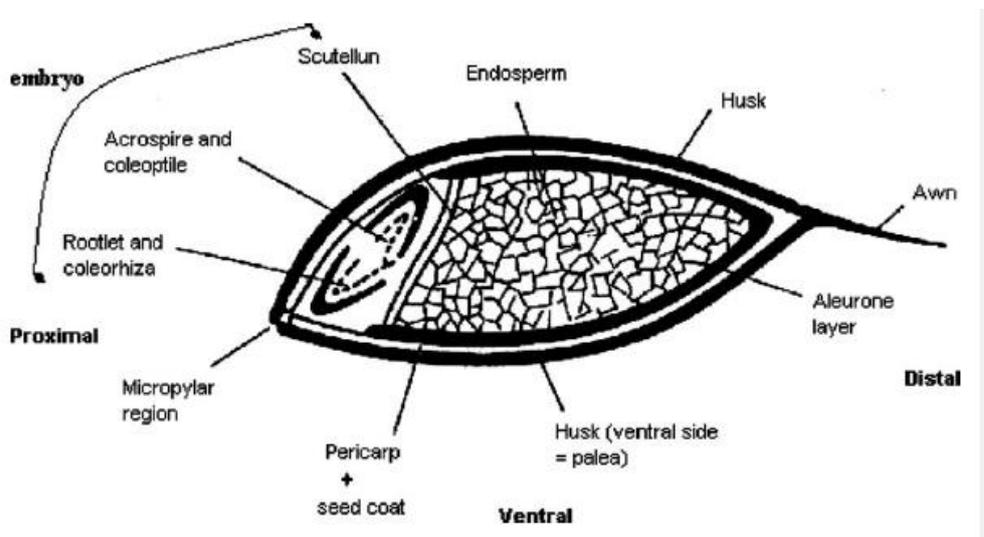
2) germination;

3) drying.

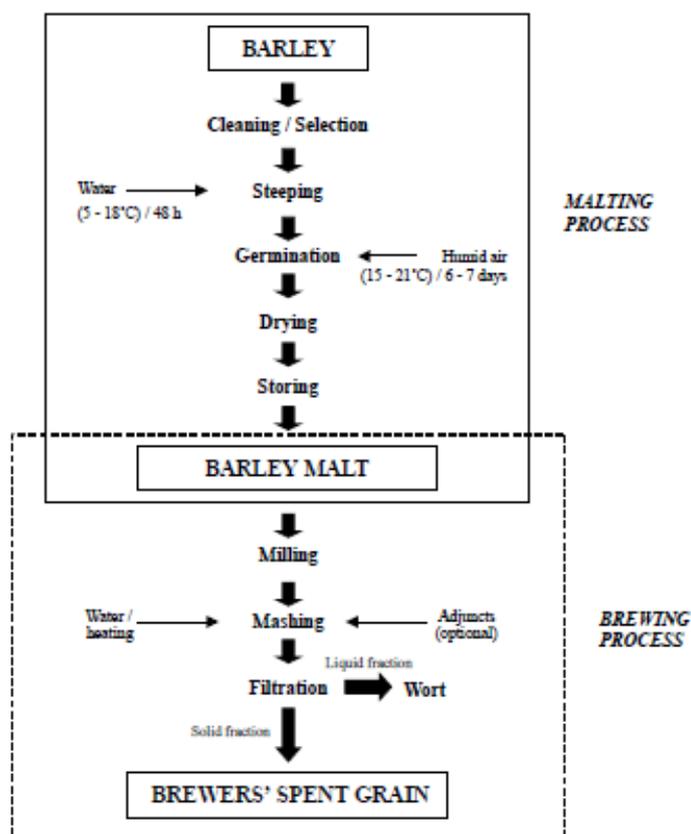
During the maceration the crucial part is the temperature of the water that have to be kept between 5-18°C for 2 days. The role of the water is enter in the embryo and rise the moisture up to 42-48% changing the water every 6-8 h. This hydration process initiates the germination phase thanks to the activation of aleurone metabolism. After maceration the grain are conveyed to a germination vessel where they are turned thanks a helix turns that allow to keep in contact with humid air the flow thought grain bed and kept at 15-21 °C. The germination process promotes the activation of the starchy enzymes that included amylases, proteases, beta-glucanases. The germination process usually lasts 6-7 days and allow the modify of starchy endosperm. The malt barley is dried at 40-60°C to a 4-5% moisture content, to avoid contamination and so spoilage by microorganisms. After this step the malt is stored to 3 or 4 weeks to reach the homogeneity ( Kendal 1994; Tschöpe, 2001; Venturini Filho and Cereda, 2001).

in the beer processing, the malted grains of barley are milled, mixed with water in the mash tun with a constantly increase of temperature ( from 37 to 78°C) this process allow the degradation ( hydrolysis) of compound such as starch ( mainly), protein, (  $1\rightarrow3$ ;  $1\rightarrow4$  )  $\beta$ - glucans and arabinoxylans. This process has as main aim the conversion of the main non fermentable sugar (starch) in to the simpler fermentable sugar ( mainly maltose and maltotriose ) but also in simpler non fermentable ( dextrans), while the protein are degraded in small polypeptides and amino acids. The result of this enzymatic process is a liquid called wort with a dispersion of solid compound that usually are collected in the bed bottom of mash tun. This sweet liquid plus solid compound dispersed is filtered, the wort is used for the production of beer, while the solid fraction is called brewers' spent grain (BSG) (Fig2.) (Dragone et al., 2002; Linko et al., 1998).

**Fig. 1:** Schematic representation of barley kernel in longitudinal section (Lewis and young, 1995)



**Fig. 2:** Schematic representation of the process to obtain BSG from natural Barley

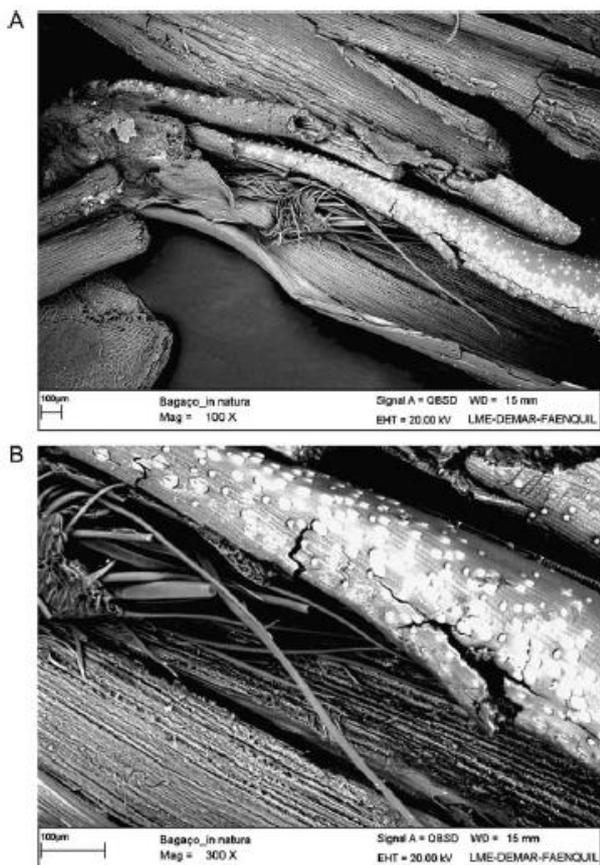


## 1.2 Composition of brewers' spent grain (BSG)

The composition of the BSG basically is given from the part involved in the protection of the raw barley grain and so: husk, pericarp and seed coat layers. The right composition of the con change for multiple reasons as the kind of beer produced, and if the original malt is mixed with other cereals( wheat rice or maize) for the operation of adjustment, for example in the lack of fermentable sugar in the wort especially in the dark beer consequent of the destruction of indigenous amylases for effect of roasting process; the barley variety; harvest time; malting and mashing condition (Reinold, 1997; Huige, 1994; Santos et al., 2003). However the major component of BSG is the walls of the the husk, pericarp and seed coat that is composed basically of cellulose and non-cellulose polysaccharides and lignin and may contain protein, lipid and silica that is present in high amount in the husk that are visible in the picture of scanning electronic microscopy (Fig. 3) (medleod, 1979). The major component as before written is cellulose and lignin so it can be consider a lignocellulosic material rich in protein and fibre, in fact it can be counted around 20- 70 % of the total material. The main components of these fibrous material are lignin, arabinoxylan and cellulose . The completed composition of BSG are showed in the (table 1) (Santos et al. 2003)

In BSG is also possible to find mineral, vitamins and amino acids. For the minerals it is possible to find: calcium, cobalt, copper, iron, magnesium, manganese, phosphorous, potassium, selenium, sodium and sulphur but each one in concentration then of 0.5% (Huge, 1994; Pomeranz and Dikeman, 1976). The vitamins include (ppm): biotin (0.1), Choline (1800), folic acid (0.2), niacin (44), pantothenic acid (8.5), riboflavin (1.5), thiamine (0.7) and pyridoxine (0.7); and for the amino acid content it is possible to find: include leucine, valine, alanine, serine, glycine, glutamic acid and aspartic acid in the largest amounts, and tyrosine, proline, threonine, arginine, and lysine in smaller amounts. Cystine, histidine, isoleucine, methionine, phenylalanine, and tryptophan can also be present (Huge, 1994; Mariani, 1953).

**fig.3:** scanning electron microscopy of BSG particles. (A) Magnification 100 fold; (B) magnification 300 fold. The bright points in the external portion of the husk are silicates



**Table 1:** Chemical composition of brewer's spent grain (BSG) and germinated barley foodstuff (GFB)

Component (% dry wt)	BSG <sup>a</sup>	BSG <sup>b</sup>	GBF <sup>a</sup>	GBF <sup>c</sup>
Cellulose	25.4	16.8	8.9	9.1
Arabinoxylan	21.8	28.4	17.0	19.2
Lignin	11.9	27.8	8.2	6.7
Protein	24.0	15.2	46.0	48.0
Lipid	10.6	Nd	10.2	9.2
Ash	2.4	4.6	2.0	2.0

<sup>a</sup> From Kanauchi et al. (2001).

<sup>b</sup> From Mussatto and Roberto (2005).

<sup>c</sup> From Fukuda et al. (2002). nd, no determined.

### 1.3 Preservation and storage

The BSG as brewery by-product has an high concentration of water around 77-81% ( w/w) ( huige, 1994; Russ et al., 2005). For this reason and the presence of fermentable sugar, is a great substrate suitable for the growth of the microorganism and consequently spoilage. For example after storage in gunnysacks for 30 days are been isolated species of *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium* and *Rhizopus* (Sodhi et al. 1995). Several method can be used to avoid this kind of problem and extend the period of storage without any kind of problem the BSG. Addition of organic acid as lactic, formic, acetic or benzoic in a mixture with water inside a plastic container and the BSG is able to overpass the three summer months preserving the original quality (Al-Hadithi et al., 1985). Another alternative is drying the biomass that improve the storage reducing the space and so also the transportation cost (Santos et al., 2003). The drying method used for the BSG are different: the traditional one with the rotary drum dries that is the most expensive in term of energy use; are been tried different treatment such as freeze- drying that resulted economically unacceptable for this kind of product, while the Freezing process is not used because doesn't reduce the space and it showed changing in the arabinose content. Oven drying of BSG that have to be conducted at temperatures lower than 60°C because higher temperature could create unwanted flavour and cause the roasting of the biomass that is not acceptable (Hernandez et al., 1999; Prentice and D' Appolonia, 1977; Huige, 1994). Another alternative method to reduce the problem of pollution and risk is with the superheated steam si filtering with special membrane and after drying under vacuum to reach 20-30% of moisture. No bacterial activity in the cakes for 6 months ( El-Shafey et al 2004, tang et al., 2004, 2005).

## 1.4 Application of BSG as animal feeding

Thanks the high nutritional value showed above in the table 1, the BSG is suitable like a good feeding material for the breeding especially of some breed of ruminant. BSG can be used both dried than immediately after filtering from the beer wort (öztürk et al., 2002; Townsley, 1979). Thanks a his characteristics BSG can be used mixed with inexpensive nitrogen sources (urea) to provide all the essential amino acids. The addition of it as feeding can increase the yield of the milk production without any kind of problem linked to the fertility of the animal (Belibasakis and Tsirogianni, 1996; Reinold, 1997; Sawadongo et al., 1989). The addition for example of BSG at the diet of cows showed an increase of milk total solid content, fat yield and general yield of the milk. This improvement does not show an increase in any kind of blood value (Belibasakis and Tsirogianni, 1996). The same happened with the fish, the substitution of with rice bran with the BSG and this give at the fish an increase in size thanks the high protein content. (kaur and Saxena 2004).

## 1.5 Application as 'human nutrition'

BSG is starting to be interesting also for human feeding this thanks his low cost and the high nutritive value. It has been used in bakery product (bread, biscuit aperitif snack), after been converted in flour, as completely substitution in a mix with the normal flour ( Hassona,1993; Miranda et al.,1994a,b; öztürk et al., 2002). Nevertheless there are some limitation to the use of this flour as additive due at the particular colour and flavour, because BSG flour has brownish colour, so it's change to much the right appearance of the product made with the normal flour, with consequent rejection of the final consumer. Total different is the situation with the product are made with wholemeal flour, but anyway to avoid any kind of unwanted alteration of the product (physical e.g. texture) only small quantities around 5-10% can be incorporated (Hassona, 1993; Miranda et al., 1994a,b; Townsley, 1979).

The addition of BSG flour in a bread with a concentration of 10% showed an increase of the protein and essential amino acid content by 50 and 10% , doubled the fibre content and reduce about 7% of calories compare with the traditional ( Hassona, 1993).

The health benefit linked with the ingestion of BSG are various: increase the fecal weight, increase cholesterol and fat excretion, in the rat it showed an beneficial in intestinal digestion (Fastnaught, 2001). Those effect are attributed to the present of glutamine-rich protein and non-cellulosic polysaccharides (arabinoxylan, 20-47%) and small amount of (1→3, 1→4) β-glucans (less than 1%) ( McCleary and Nurthen, 1986; Vietor et al., 1993).

The increasing interest of the consumer to the health life created a big challenge in the past years, the use of BGS as substitution of fat component in particular meat product. Especially in product with low nutritional value as additive like in sausages and frankfurters. It's know that various disease are linked with an high consumption of animal fat like coronary heart disease, obesity, cancer (Ozvural and vural, 2008).

however the creation of this meat sausages without the presence of fat it's impossible, in fact the percentage of the fat in traditional emulsion of sausages is up to 30% (Choi et al., 2009). It's plays an important role in the characteristic of the final product, the shape, the cooking yield, flavour, an especially the texture of the final product (Choi et al. 2010; Youssef and Barbut, 2009).

The previous studies in this field showed that the replacement of animal fat with a fibre increase the final value of the product, because the final product has a lower calories, the introduction of fibre in a meat emulsification increase the stabilization of the product.

## **1.6 Advantage of BSG addiction in meat product**

The composition of BSG as mentioned above is rich in fibre, that is very important In the human diet. Addition of BSG in the meat product as substitution of meat or fat is increasing year by year because this substitution allow at the final product to gain special functional proprieties both nutritional than processing. For what concern the nutritional features the BSG is considered a source of fiber and the intake of it help the regulation of serum cholesterol and low-density of fatty acids, reduction of diabetes and cancer risk and help the colonization of the right gut microflora ( Burkitt, 1971; Painter & Burkitt, 1971; Trowell, 1972; Walker et al., 1973; Anderson et al., 1990; Schneeman, 1999; Izydorczyk et al., 2001), in addition the substitution of BSG with the fat component of sausages reduce the kcal of the product. This last aspect interest also some functional properties during the processing as reduction of the amount of product during the cooking and modification of texture, colour, and flavour that result be the main challenges to win for the final appreciation of the consumers. Another important skill that the sausages gain with the addition of BSG is the increase of the water hold capacity (Salama et al., 1995; Jimenez-Colomenero 1996; Akoh 1998).

## **1.7 Pork meat**

Meat is one of the most common food consumed in the world. Going deeper the most used meat result be the pork meat, in fact according with the USDA's statistics pork is in the first position with the 43% of the total meat consumption followed by poultry 27% and beef 26% and only 4% of others kind of meat ([www.omafra.gov.on.ca](http://www.omafra.gov.on.ca)). The situation of course is the same moving the focus on the European Union and the prevision is that the consumption will in increase in relation with the growth of the human population in the world that is strictly correlated with the higher meat consumption (Pierzchała *et al.*, 2006).

The high demand of pork affect directly the quality of it because to achieve the demand level the farmer are pushed to make an always more intensive production that of course bring the drop down of the quality. In the other hand the consumers are moving to care avout the quality of meat. The consumer's quality is given principally to the appearance characteristics as shape and colour, the sensorial features as odour and taste and the easy handling (Monin, 1998; Verbeke et al., 2005; Fortomaris et al., 2006, Ngapo et al., 2007a).

The composition of the meat pork is an aspect strictly linked with the quality of each final product made with this raw material. The component that divide the pork meat for quality are the fat (intra and intercellular) and the muscle part. Those part on animals are linked with several aspect during the animal life as species of the pigs, different technique of breeding, different feeding product.( Żak and Różycki 2008). In particular for this study was used the loin cuts from the pig, it's considered one of the best pork cut (Virgilli et al. 2003, Winiarski et al. 2004). The proportion fat-to-muscle determines the quality of this cut that can be used for different purpose. When the meat taken in account is from pork it's necessary do a clarification because is known that a low content of fat, especially intramuscular are related with a worse taste of the cuts (Faucitano *et al.* 2003).

## 2 Materials and methods

### 2.1 Preparation of BSG

Brewer's spent grain (BSG) was obtained brewery production section inside the university (Wrocław University of environmental and life science ).

It was used two kind of BSG and the difference was the temperature of drying, the BSG 13 was dried in an electric oven at 55°C and the second, BSG 14 at 60 °C to reach for both the moisture content less than 10 %. The dried BSG was milled using a laboratory grinder and packed in polyethylene bags and stored in refrigeration temperature 4°C until the use. During this investigation was use the BSG 14.

### 2.2 Sausages preparation

The meat was obtained to a common slaughterhouse. The meat used was got, both the fat and the meat, from the loin cut of the pork.

The meat obtained was milled with a commercial meat grinder and mixed with the species following the recipe on Tab.2

**Table 2:** Pork sausages formulations of the different samples: control, BSG (brewer's spent grain) 2%, 4%, 6% and 8 %.

	<b>Control</b>	<b>BSG 2%</b>	<b>BSG 4%</b>	<b>BSG 6%</b>	<b>BSG 8%</b>
<b>Meat</b>	24,53 g	24,53 g	24,53 g	24,53 g	24,53 g
<b>Fat</b>	12,3 g	11,39 g	10,49 g	9,6 g	8,7 g
<b>ice</b>	8,19 g	8,19 g	8,19 g	8,19 g	8,19 g
<b>Curing salt</b>	0,855 g	0,855 g	0,855 g	0,855 g	0,855 g
<b>pepper</b>	0,0675 g	0,0675 g	0,0675 g	0,0675 g	0,0675 g
<b>Nutmeg</b>	0,0225 g	0,0225 g	0,0225 g	0,0225 g	0,0225 g
<b>BSG</b>	0	0,9 g	1,8 g	2,7 g	3,6 g
<b>Total weight</b>	45g	45g	45g	45g	45g

The normal recipe was used as a control, while was prepared other 4 recipes with the addition of 2%, 4%, 6% and 8% of BSG. The percentage was referred a the total weight of the original recipe, the substitution of meat with the BSG was, in particular, focused into the fat fraction. After the preparation of the meat with the different amount of BSG, with the preparation was filled the falcon tube and the samples were cooked in the boiling bath and consequently stored in the chilling room at 4°C before the analysis.

## 2.3 Sausages analysis

### 2.3.1 Proximate Analysis and pH

The procedures and measurements for the proximate analysis and pH were the following: the pH was directly measured in the sample in triplicate way using a pH meter electrode (Orion 3-Star pH Benchtop Meter, Thermo Fisher Scientific Inc.) at room temperature; the moisture content was evaluated in duplicate according to AOAC 2005, 950.46; so, dried samples from moisture analysis with the electronic oven at a temperature of 105°C, so subjected to ether-extraction using the Soxhlet apparatus following the AOAC 960.39 extraction method; (FOSS, Hilleroed, Denmark). All the proximate analysis were performed in duplicate (n=2) during the first and the second week of storage, while the pH was evaluate also for the third week of storage.

### 2.3.2 Sensory analysis

All the sample were assessed on the first and second week of storage. The sausages were cut in smaller slices with a dimension of 1 cm, at each sample evaluated was assigned a hidden code without showing the percentage of BSG and so evaluated at room temperature. The evaluation was effectuated by eight non-expert panelists (n=8) using a five-point hedonic rating scale of acceptance (from 1 – extremely dislikeable to 5- extremely likeable) (Lawless & Heymann, 2010).The parameters analysed in the final sausages were the following: smell, appearance, colour, taste, texture, consistency, fibreness and juiciness.

### 2.3.3 Colour

The colour parameters of the sausages were determined by the CIE Lab system: L (lightness, a (redness) and b (yellowness). The measurement was repeated five times by using a reflectance colorimeter (Minolta CR-400), on a special slice of sausages 15x25 mm. All the samples were measured five times each week of storage (first, second and third week).

### 2.3.4 Texture profile analysis

The texture profile analysis (TPA) was performed making five repetitions ( n=5) using Zwick/Roell Z010 testing machine (Zwick Testing Machines Ltd., Leominster Herefordshire, UK). The samples used were cut in a slice of 10x25 mm, and consequently compressed twice to 50% of the original shape to evaluate the following parameters (Salejda, et al,2016):

- Cohesiveness: the ratio between the active work for the second compression to the work necessary for the first compression ;

- Elasticity;
- Gumminess: energy necessary to destroy a semisolid food in a way that can be swallowed ;
- Chewiness: work necessary to chew the sample.

The TPA analysis of all the samples was evaluated for five-time each sample at room temperature on the first, second and third week of storage.

### **2.3.5 Thiobarbituric reactive substances (TBARS)**

The thiobarbituric reactive substances was determined using the following method :

- Take 0,5g of sausages meat, add 5 ml 10 % TCA solution
- Homogenize with hand blender for 1 minute
- At same time start to boiling a container full of water
- Transfer to a centrifuge tube and centrifuge at 4000 x g for 10 minutes
- Take 2 ml supernatant, add 2 ml 0.02 M thiobarbituric acid
- Heat in a boiling water bath for 40 minutes
- Cool in a cold water ( ice ) for 2 minutes
- Take measurement with spectrophotometer at wave length of 530 nm

The result were expressed as mg malondialdehyde per kg of sample. Absorbance (A) were used to calculate the TBA value as ( mg malondialdehyde /kg treatment) with this formula :  $7,8 \cdot A$  (Vural and Öztan, 1996).

### **2.3.6 Data analysis**

The obtained data were analysed using parametric analysis of variance (ANOVA) followed by Tukey's HSD post-hoc test was applied to compare the means at the level of confidence of 95 % ( $p < 0.05$ ). Conversely, when the normality of the distribution and the homogeneity of the variances were not satisfied, non-parametric ANOVA (Kruskal-Wallis) along with Holm's post-hoc tests were carried out at the same level of significance ( $p < 0.05$ ). R statistical software (R foundation for statistical computing, Vienna, Austria) was employed to perform all the tests.

## 3 Results and discussion

### 3.1 Proximate analysis and pH

#### 3.1.1 Lipid content

Control and treated pork sausage samples were analysed and is reported in Table 3.

The lipids content analysis was evaluated only for the first two weeks, from the results was observed that there are not significant differences between the control and the treated samples, however, it is observed a progressively decreasing of lipids by increasing the amount of BSG. The reason of this trend is given by the physically replacement of the pork fat with the BSG. For the second week of storage, the evaluation was measured only between the treated samples with BSG. In general, for sample treated with the higher concentration of BSG a significantly decrease was observed compared to the other treated sausages. However, the pork sausage enriched with 2 % of BSG reported the highest lipid content around 19.37 %, which was similar to the control sausage in the first week. Considering the time parameter from the table it observed the slight decreased of lipids, could be due to the human error during the handling for preparation of the sample analysed.

According with Özvural et al. (2009) which investigated the utilization of brewer's spent grain (1%,3%,5%) in the production of Frankfurters even if considering the different meat and fat (beef).

The total fat content of the control sausage (28.6 %), which had the highest added fat in the formulation, was higher compared to the sausages enriched with fibres. Fat content was similar amongst fibre containing sausages ranging between 15.2 % and 16.0 %. These lower total fat values were attributed to the low amounts of fat added in the dietary fibres-sausage formulations (Henning et al., 2016).

**Table 3:** Lipid content (%) of the control and treated pork sausages samples with different concentrations of BSG analysed during the first and second storage week.

	lipids[%]	
	first week	second week
<b>control</b>	19,12±0,20 <sup>a</sup>	ND
<b>2% BSG</b>	18,85±0,36 <sup>a</sup>	19,37±2,43 <sup>a</sup>
<b>4% BSG</b>	16,96±0,57 <sup>a</sup>	13,96±0,16 <sup>ab</sup>
<b>6% BSG</b>	17,41±0,66 <sup>a</sup>	14,46±0,19 <sup>bc</sup>
<b>8% BSG</b>	16,02±0,79 <sup>a</sup>	15,35±0,42 <sup>c</sup>

Different letters indicate significant differences ( $p < 0.05$ ) between samples at each week of storage.

### 3.1.2 Protein content

The protein content analysis was evaluated only for the first two weeks of storage. Results related to the protein content for control and treated pork sausages were reported in Table 4. Considering the obtained results there were not significant differences between the control and the treated sample with BSG. The same behaviour was observed for the second week of storage for all the pork sausages samples treated with BSG. The results of the protein content was not expected, considering the high content of protein in the BSG, but this could be due to the low percentage of product added in the formulation of the sausages. In fact according with Ktenioudaki et al. (2012) which investigated in Brewer's Spent Grain (15%, 25%, 35%) as a Functional Ingredient for Breadsticks showed a significant increase of protein percentage. Henning et al. (2016) reported an higher increase of the protein content on the control sausage (around 16.2%) compared to the other samples which contained different pineapple dietary fibres (1%) ranging from 12.8 to 13.9%. This had been explained by the availability of meat protein in the added fat (control), which was highly reduced in the sausage samples containing fibres.

**Table 4:** Protein content (%) of the control and treated pork sausages samples with different concentrations of BSG analysed during the first and second storage week.

	protein	
	first week	second week
<b>control</b>	14,91±0,36 <sup>a</sup>	ND
<b>2% BSG</b>	15,50±0,05 <sup>a</sup>	15,51±0,66 <sup>a</sup>
<b>4% BSG</b>	14,47±0,08 <sup>a</sup>	14,83±0,08 <sup>a</sup>
<b>6% BSG</b>	14,79±0,01 <sup>a</sup>	15,21±0,09 <sup>a</sup>
<b>8% BSG</b>	14,81±0,48 <sup>a</sup>	15,24±0,30 <sup>a</sup>

Different letters indicate significant differences ( $p < 0.05$ ) between samples at each week of storage.

### 3.1.3 Moisture content

The moisture content analysis was evaluated only for the first two weeks of storage. Results related to the moisture content for control and treated pork sausages were reported in Table 5. In the first week any significant variations were observed among the different samples. The same trend was reported also for treated sausages samples during the second week of storage.

Özvural et al. (2009) showed a different trend with consequent decrease of moisture content increasing the BSG content, this is probably due to the different formulation of sausages in term of ratio of lipid-BSG repletion, starch addition and meat used (beef) factors that increase considerably the water hold capacity.

**Table 5:** Moisture content (%) of the control and treated pork sausages samples with different concentrations of BSG analysed during the first and second storage week.

	moisture[%]	
	First week	second week
<b>control</b>	64,70±0,15 <sup>a</sup>	ND
<b>2% BSG</b>	64,51±0,05 <sup>a</sup>	64,88±0,30 <sup>a</sup>
<b>4% BSG</b>	64,90±0,44 <sup>a</sup>	66,85±0,10 <sup>a</sup>
<b>6% BSG</b>	63,91±0,73 <sup>a</sup>	64,66±0,72 <sup>a</sup>
<b>8% BSG</b>	62,88±1,32 <sup>a</sup>	62,51±0,26 <sup>a</sup>

Different letters indicate significant differences ( $p < 0.05$ ) between samples at each week of storage.

### 3.1.4 PH

The pH evaluation was investigated for 3 weeks of storage. It showed no significant differences between the control and the different BSG samples during the first week. However, in the 2nd and 3rd weeks the evaluation was made only between the BSG treated samples and also in this case, in the week there weren't observed significant differences.

In accordance with Özvural et al. (2009) the results showed the same trend of the literature. However, Henning et al. (2016) observed a reducing pH for different species sausages (containing pork, beef and sheep) around 4.8-4.9 enriched with pineapple dietary fibres (1 %) compared to the control (around 5).

**Table 6:** pH measurements of the control and treated pork sausages samples with different concentrations of BSG analysed during the first, second and third storage week.

	PH		
	first week	second week	third week
<b>control</b>	6,15±0,05 <sup>a</sup>	ND	ND
<b>2% BSG</b>	6,20±0,05 <sup>a</sup>	5,18±0,32 <sup>a</sup>	6,22±0,12a
<b>4% BSG</b>	6,37±0,29 <sup>a</sup>	5,40±0,36 <sup>a</sup>	5,50±1,01a
<b>6% BSG</b>	6,03±0,02 <sup>ab</sup>	5,42±0,63 <sup>a</sup>	6,10±0,14a
<b>8% BSG</b>	5,95±0,05 <sup>b</sup>	5,59±0,26 <sup>a</sup>	6,06±0,07a

Different letters indicate significant differences ( $p < 0.05$ ) between samples at each week of storage.

### 3.2 Sensory analysis

The sensorial analysis was evaluated only for the first 2 weeks. The obtained results were reported in Table 7. The results in the first week did not showed significant differences between the control and the BSG treated samples at any percentage of addition for each sensorial parameter : smell, appearance, colour, taste, texture, consistency, fibreness. The trend was confirmed also in the second week for exception of the smell parameter that showed a progressively decrease of appreciation with the increase of BSG amount added and for the juiciness that showed the same trend. Nevertheless, the results showed not a big difference between the samples, it was a great result because it mean that there were not big differences between the control and the samples treated with BSG in term of appreciation, but it could be also due to the use of a non-trained and exert panellists.

Moreover, sensory characteristics are strongly dependent on the used ingredients on the mixture formulation of sausages. In fact, Özvural & Vural (2011) observed that the addiction of grape seed flour, from wine by-products, incorporated into frankfurters at seven concentrations (0, 0.5, 1, 2, 3, 4, and 5%) provoked an effect on the palatability of the product. An amount of grape flour above 0.5% reduced the overall acceptability, while the frankfurters containing the level up to 2% received scores above the average.

**Table 7:** Sensory results of the control and treated pork sausages samples with different concentrations of BSG analysed during the first and second storage week.

		smell	appearance	colour	taste	texture	consistency	fibreness	juiciness
first week	control	2,57±1,27 <sup>a</sup>	2,13±1,25 <sup>a</sup>	2,25±1,49 <sup>a</sup>	2,88±0,83 <sup>a</sup>	2,75±1,04 <sup>a</sup>	3,00±1,07 <sup>a</sup>	3,25±0,71 <sup>a</sup>	3,13±1,46 <sup>a</sup>
	2% BSG	3,13±0,83 <sup>a</sup>	3,00±0,76 <sup>a</sup>	3,00±0,53 <sup>a</sup>	3,50±1,07 <sup>a</sup>	3,13±0,83 <sup>a</sup>	2,88±0,83 <sup>a</sup>	3,25±1,04 <sup>a</sup>	3,50±0,76 <sup>a</sup>
	4% BSG	3,25±0,71 <sup>a</sup>	3,88±0,83 <sup>a</sup>	3,50±1,07 <sup>a</sup>	3,25±1,04 <sup>a</sup>	3,38±1,06 <sup>a</sup>	3,50±1,20 <sup>a</sup>	3,00±0,93 <sup>a</sup>	3,38±0,92 <sup>a</sup>
	6% BSG	2,63±1,06 <sup>a</sup>	3,00±0,76 <sup>a</sup>	3,25±0,89 <sup>a</sup>	3,00±1,07 <sup>a</sup>	3,25±0,71 <sup>a</sup>	3,25±0,89 <sup>a</sup>	2,75±1,04 <sup>a</sup>	3,13±0,99 <sup>a</sup>
	8% BSG	2,38±1,41 <sup>a</sup>	3,13±1,25 <sup>a</sup>	3,13±0,83 <sup>a</sup>	2,50±1,20 <sup>a</sup>	3,00±1,31 <sup>a</sup>	3,13±1,36 <sup>a</sup>	3,00±1,51 <sup>a</sup>	3,25±1,28 <sup>a</sup>
second week	control	3,71±0,76 <sup>a</sup>	3,71±1,25 <sup>a</sup>	3,57±1,27 <sup>a</sup>	3,43±0,98 <sup>a</sup>	3,86±0,69 <sup>a</sup>	3,71±0,49 <sup>a</sup>	2,86±1,07 <sup>a</sup>	3,71±0,49 <sup>a</sup>
	2% BSG	3,25±0,46 <sup>ab</sup>	3,63±1,06 <sup>a</sup>	3,50±0,76 <sup>a</sup>	3,38±0,74 <sup>a</sup>	3,75±0,46 <sup>a</sup>	3,38±0,52 <sup>a</sup>	3,38±0,52 <sup>a</sup>	3,13±0,35 <sup>ab</sup>
	4% BSG	2,50±1,07 <sup>bc</sup>	3,25±1,28 <sup>a</sup>	3,63±0,92 <sup>a</sup>	2,75±1,28 <sup>a</sup>	3,25±1,16 <sup>a</sup>	3,13±1,13 <sup>a</sup>	3,25±0,89 <sup>a</sup>	2,88±0,83 <sup>abc</sup>
	6% BSG	2,38±1,19 <sup>bc</sup>	3,13±0,99 <sup>a</sup>	3,38±0,74 <sup>a</sup>	3,25±0,71 <sup>a</sup>	3,38±0,52 <sup>a</sup>	3,50±0,53	3,50±0,93 <sup>a</sup>	3,00±0,76 <sup>bc</sup>
	8% BSG	2,25±0,89 <sup>c</sup>	2,75±1,28 <sup>a</sup>	2,38±1,19 <sup>a</sup>	2,63±0,52 <sup>a</sup>	2,88±0,64 <sup>a</sup>	2,75±0,46 <sup>a</sup>	3,00±1,20 <sup>a</sup>	2,25±0,89 <sup>c</sup>

Different letters indicate significant differences ( $p < 0.05$ ) between samples at each week of storage.

### 3.3 Colour

The colour analysis showed a progressively and significant decreasing of lightness ( $L^*$ ) between the control and the treated samples with BSG as reported in the table in fact the highest  $L^*$  parameter was observed for the control (71,4) while the lowest was 8% BSG which had 61,5 during the first week of storage. The proportionally increased of BSG determined the also the decrease of  $a^*$  and an increase of  $b^*$  parameter compared to the control during the first week of storage. However, for the 2nd and the 3rd week the treated samples were compared between each other. In this way, the pork sausages considering the  $L^*$  parameter follow the same trend as the first week but the parameter time of storage showed a further decrease between the samples. The first week trend was respected also in the  $a^*$  and  $b^*$  parameters but the time influence showed an increase of  $a^*$  while  $b^*$  remain constant.

The difference of those parameters was probably for different reasons, considering the changing between the control and the treated samples, in the same week. The reason was the natural colour of BSG that could be determined yellowish/brownish, so the progressively adding of it into the pork sausages with a range of colour between pink to reddish leads a loss of lightness ( $L^*$ ) and the perception of redness ( $a^*$ ). While the reported increase of  $b^*$  parameter could be reflected with an increase of the yellowness perception. In addition, the differences in the light scattering properties of the different sausage formulations could be the cause of the different colour parameters between the control and the brewer's spent grain containing sausages (Varnam & Sutherland, 1995).

In according with Choi, et.al, (2014), which investigated the replacing of chicken back fat with brewer's spent grain (approximately 20, 25, and 30%) in chicken sausages, similar results related to  $L^*$  and  $b^*$  values while for  $a^*$  parameter a progressively increase were observed compared to non-treated sausages, this could be due to the different used raw matrix (chicken instead of pork).

**Table 8:** Colour results of control and treated pork sausages samples with different concentrations of BSG analysed during the first, second and third storage week.

		<b>L*</b>	<b>a*</b>	<b>b*</b>
<b>first week</b>	<b>control</b>	71,4±1,1 <sup>a</sup>	5,8±0,3 <sup>a</sup>	7,4±0,3 <sup>a</sup>
	<b>2% BSG</b>	65,7±2,6 <sup>b</sup>	5,6±0,4 <sup>a</sup>	7,9±0,7 <sup>a</sup>
	<b>4% BSG</b>	64,8±1,1 <sup>bc</sup>	5,1±0,1 <sup>ab</sup>	9,2±0,3 <sup>a</sup>
	<b>6% BSG</b>	63,5±0,3 <sup>cd</sup>	5,4±0,2 <sup>bc</sup>	10,2±0,3 <sup>a</sup>
	<b>8% BSG</b>	61,5±1,5 <sup>d</sup>	4,8±0,3 <sup>c</sup>	11,7±0,8 <sup>a</sup>
<b>second week</b>	<b>2% BSG</b>	68,4±1,1 <sup>a</sup>	5,9±0,2 <sup>a</sup>	8,3±0,2 <sup>a</sup>
	<b>4% BSG</b>	64,5±0,7 <sup>a</sup>	5,5±0,2 <sup>ab</sup>	9,3±0,3 <sup>b</sup>
	<b>6% BSG</b>	64,5±0,6 <sup>b</sup>	5,3±0,1 <sup>bc</sup>	10,2±0,1 <sup>c</sup>
	<b>8% BSG</b>	59,9±1,8 <sup>c</sup>	4,9±0,2 <sup>c</sup>	11,8±0,6 <sup>d</sup>
<b>third week</b>	<b>2% BSG</b>	66,9±0,2 <sup>a</sup>	6,3±0,2 <sup>a</sup>	7,9±0,1 <sup>a</sup>
	<b>4% BSG</b>	65,1±1,1 <sup>ab</sup>	5,4±0,3 <sup>b</sup>	9,1±0,0 <sup>a</sup>
	<b>6% BSG</b>	62,3±0,6 <sup>c</sup>	5,4±0,2 <sup>b</sup>	9,8±0,3 <sup>a</sup>
	<b>8% BSG</b>	62,8±0,8 <sup>c</sup>	5,0±0,0 <sup>c</sup>	10,9±0,1 <sup>a</sup>

Different letters indicate significant differences ( $p < 0.05$ ) between samples at each week of storage at each week of storage.

### 3.4 Texture profile analysis

The TPA analysis was evaluated for three weeks of storage. Results related to the different measured texture parameters were shown in Table 9. For the first week, the control was compared to the treated BSG sausages samples, while in the other two weeks only the treated samples were analysed. As observed, the results in the first week showed significant differences between the samples. Considering the elasticity parameter, the value decreased progressively by increasing the BSG % in the sausages, with the unique exception of the 4% BSG, which did not show differences with the control. The same trend was followed for the other parameters: gumminess, chewiness, and cohesiveness, with the same exception of the 4% BSG-enriched sample. This fact probably means that the progressive increase of BSG in the pork sausages changes the texture of the product. Moreover, the BSG addition decreased the elasticity, gumminess, chewiness, and the cohesiveness values in a proportionally way on the amount added. The unexpected results obtained for the 4% BSG could be due to human error during the sample preparation (bigger dimension of the slice analysed and not correct homogenization of the sample with consequent different concentration of BSG material into the sausages). The same trend was shown for the second and third weeks of studying, which means that the time parameter does not affect the texture profile of the sausages.

The results obtained are in accordance with Özvural et al. (2009), which investigated the utilization of brewer's spent grain (1%, 3%, 5%) in the production of Frankfurters, even if the investigated product contained different based ingredients on the formulation (meat and fat were from beef).

**Table 9:** Texture profile analysis results of the control and treated pork sausages samples with different concentrations of BSG analysed during the first, second and third storage week.

		elasticity	Gumminess( N)	Chewiness(N)	Cohesiveness
<b>first week</b>	<b>control</b>	0,86±0,04 <sup>a</sup>	26,41±2,78 <sup>a</sup>	22,71±3,08 <sup>a</sup>	0,62±0,02 <sup>a</sup>
	<b>2% BSG</b>	0,83±0,03 <sup>a</sup>	23,44±2,01 <sup>a</sup>	19,51±2,15 <sup>a</sup>	0,58±0,03 <sup>ab</sup>
	<b>4% BSG</b>	0,86±0,03 <sup>ab</sup>	26,42±0,95 <sup>ab</sup>	22,62±1,22 <sup>ab</sup>	0,56±0,04 <sup>abc</sup>
	<b>6% BSG</b>	0,80±0,02 <sup>bc</sup>	25,81±2,71 <sup>ab</sup>	20,63±2,43 <sup>ab</sup>	0,50±0,01 <sup>bc</sup>
	<b>8% BSG</b>	0,74±0,04 <sup>c</sup>	21,74±2,22 <sup>b</sup>	16,02±2,19 <sup>b</sup>	0,50±0,08 <sup>c</sup>
<b>second week</b>	<b>2% BSG</b>	0,82±0,01 <sup>a</sup>	22,77±2,09 <sup>a</sup>	18,58±1,55 <sup>a</sup>	0,54±0,02 <sup>a</sup>
	<b>4% BSG</b>	0,84±0,03 <sup>a</sup>	22,93±7,47 <sup>a</sup>	19,46±6,72 <sup>a</sup>	0,54±0,04 <sup>a</sup>
	<b>6% BSG</b>	0,81±0,01 <sup>a</sup>	20,09±1,33 <sup>a</sup>	16,21±1,10 <sup>a</sup>	0,41±0,02 <sup>a</sup>
	<b>8% BSG</b>	0,67±0,06 <sup>b</sup>	15,10±3,13 <sup>a</sup>	10,21±2,78 <sup>a</sup>	0,50±0,03 <sup>b</sup>
<b>third week</b>	<b>2% BSG</b>	0,80±0,04 <sup>a</sup>	20,60±3,80 <sup>a</sup>	16,69±3,45 <sup>a</sup>	0,50±0,03 <sup>a</sup>
	<b>4% BSG</b>	0,83±0,01 <sup>ab</sup>	23,48±2,94 <sup>a</sup>	19,57±2,68 <sup>a</sup>	0,46±0,07 <sup>ab</sup>
	<b>6% BSG</b>	0,80±0,02 <sup>ab</sup>	21,38±1,33 <sup>a</sup>	17,02±1,32 <sup>a</sup>	0,43±0,02 <sup>ab</sup>
	<b>8% BSG</b>	0,76±0,03 <sup>b</sup>	19,51±3,94 <sup>a</sup>	15,00±3,39 <sup>a</sup>	0,44±0,02 <sup>b</sup>

Different letters indicate significant differences (p<0.05) between samples at each week of storage.

### 3.5 TBARS analysis

TBARS analysis measure the oxidation process of fatty acids, when the oxidation happen the hydroperoxides of polyunsaturated fatty acids in the meat became malondialdehyde, so the TBARS analysis was performed to quantify this product of oxidation (Stefanello et. al, 2015).

The result showed a slightly decrease of the oxidation process in the samples treated with the BSG with the lowest value with the 6 % BSG composition during the first week. In the second week, the results did not showed any significant difference as well in the third. Considering the time of storage the results showed the trend of the oxidation process, showing a low activity in the first and in the third week, conversely an enhanced oxidation was observed during the second week. This probably due to the fact that in the first week the oxidation was starting, while during the second week the oxidation achieved the maximum rate and level of reactive compound, finally in the third sausages samples reached the minimum level that means that the oxidation was already ended.

Stefanello et. al (2015) which investigated the oxidative and microbiological stability of fresh pork sausages with added sun mushroom powder showed similar results.

The utilization of grape seed flour at different concentration (0, 0.5, 1, 2, 3, 4, and 5%) in frankfurters led to a decline in the oxidation level of the products, probably due to its antioxidant content rich in anthocyanin and polyphenols (Özvural &Vural 2011).

**Table 10:** TBARS values of the control and treated pork sausages samples with different concentrations of BSG analysed during the first, second and third storage week.

	TBARS [mg malondialdehyde/kg]		
	first week	second week	third week
<b>control</b>	0,42±0,07 <sup>a</sup>	1,93±0,05 <sup>a</sup>	ND
<b>2% BSG</b>	0,31±0,06 <sup>ab</sup>	1,88±0,34 <sup>a</sup>	0,01±0,02 <sup>a</sup>
<b>4% BSG</b>	0,36±0,03 <sup>ab</sup>	1,98±0,46 <sup>a</sup>	0,18±0,16 <sup>a</sup>
<b>6% BSG</b>	0,23±0,07 <sup>ab</sup>	1,54±0,18 <sup>a</sup>	0,18±0,25 <sup>a</sup>
<b>8% BSG</b>	0,31±0,06 <sup>b</sup>	2,08±0,57 <sup>a</sup>	0,40±0,25 <sup>a</sup>

Different letters indicate significant differences ( $p < 0.05$ ) between samples at each week of storage.

## 4 Conclusions

The high nutritional value of BSG, the low cost, and the possibility to use waste as new food or as additive for food make it a very attractive product that could be spread along all the food chain (feeding the animal, using as additive or directly used as food).

This investigation showed how pork sausages enriched with BSG could be used as meat replacement in meat production. The enriched pork sausages improved the nutritional and technological properties such as the protein and sensorial attributes, the latter ones showed good appreciation of them. For what concern the protein content, pH and moisture content any differences were observed between the control and treated sausages samples. However, the proportionally increased of BSG determined also the decrease of  $L^*$  and  $a^*$  parameters and an increase of  $b^*$  values compared to the control during the first week of storage. In addition, the progressively increase of BSG in the pork sausages changes the texture of product, by decreasing the elasticity, gumminess, chewiness, and the cohesiveness values in a proportionally way on the amount added of BSG.

In conclusion, the BSG could be a valid resource as protein source that could be used to cover a possible lack food in the future, in order to re-use food by-product and contribute to the sustainability.

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