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# Biotransformation of brewer's spent grain and application as a food ingredient in extruded breakfast cereals

Master's thesis in Biology and biological engineering


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Cover:
Extruded breakfast cereal prototypes containing 30% fermented Brewer's spent grains, s 11.

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# Abstract

Brewers' spent grain (BSG) is a beer brewing by-product, that mainly consists of fibers (more than 50%) and proteins (up to 30%). BSG has, due to its content, a big potential to be used in the food industry but it is currently used as animal feed or discarded. To improve BSG's usability as a food ingredient bioprocessing with slime producing lactic acid bacteria has been used in this project. Incorporation of fermented BSG as a food ingredient in breakfast cereals has been performed with extrusion method. A process and recipe have been developed to produce breakfast cereal prototypes. Evaluations of the taste and texture of the breakfast cereal prototypes have been performed with texture measurements but mainly with consumer panels (focus groups).

The final breakfast cereal prototypes produced for the last focus group were all with 30% fermented BSG. The BSG were from three different breweries, Dugges, Senson and Peroni, and the fermentation was performed with and without slime production by the lactic acid bacteria. The most preferred breakfast cereal prototype was with BSG from the brewery Dugges and fermented with slime producing lactic acid bacteria.

The conclusion of this master thesis project was that it is possible to make breakfast cereal products with a content of 30% fermented BSG with extrusion method with a pleasant taste and texture.


**Keywords: Brewers spent grains, Extrusion, Breakfast cereals, Fermentation, Lactic Acid Bacteria**

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## Content

# 1 Introduction

This master thesis project was a part of FUNBREW project, a SUSFOOD project with the title *Biotransformation of brewers´ spent grain: increased functionality for novel food applications*, which is a collaboration between researchers at the University of Helsinki in Finland, University of Bari in Italy, University of Bozen in Italy and RISE Research Institutes of Sweden in Sweden. This master thesis project was performed at RISE Research Institute of Sweden.

## 1.1 Background

Brewers' spent grain (BSG) is a beer brewing by-product, which contributes to around 85% of the total by-products generated from beer brewing. BSG is insoluble and is separated from the mash before fermentation of the starch into beer [1]. 100-130 kg wet BSG (70-80% water) is obtained from 100 kg malt, which is 21-22 kg BSG per 100 liters of brewed beer [2]. BSG mainly consists of fibers (more than 50%) and proteins (up to 30%). BSG has, due to its content, a big potential to be used in the food industry but is currently used as animal feed or discarded. The average global production is estimated to 39 million tons and no recycling solution exists currently on a large scale [1].

To improve BSG's usability as a food ingredient bioprocessing with microorganisms, like fermentation by lactic acid bacteria (LAB), can be used. This can change and improve BSG as a food ingredient by enabling the production of functional compounds and change its structure. BSG mainly consists of barley husks and parts of the pericarp and seed coat layers. The proteins are mainly capsuled in cell walls and are therefore not bioavailable for humans [2]. By fermenting with LAB the cell walls can be degraded, and the proteins can be released and become more bioavailable. Bioprocessing could also increase the bioavailability of functional compounds, like phenolic compounds and antioxidants, in fiber-rich food. These compounds are bound in lignocellulosic fractions but can be released by bioprocessing. Enzymes like cellulases, xylanases and ferulic acid esterase can degrade lignocellulosic and carbohydrate fractions of BSG [1].

The LAB used in this project were *Weissella confusa A16* and *Leuconstoc pseudomesenteroides 20193*. *Weissella confusa A16* will in this report be referred to as LAB A, and *Leuconstoc pseudomesenteroides 20193* as LAB B. LAB A and LAB B are gram-positive, rod-shaped, aerotolerant anaerobes and acid-tolerant bacteria. Both bacteria produce slime when grown with sucrose. The slime might have beneficial properties that could help to bind together the product and bind water in the product which could make the extrusion physically easier. The slime mainly consists of dextran. Dextran is bacterial polyglucan constituted of chains of glucose units connected by alpha-(1-6) linkages. The synthesis of dextran is catalyzed by the enzyme dextransucrase and is extracellularly synthesized. Dextran is currently used in the food industry as a thickener in products like ice cream or jam. It can in foods improve moisture retention, prevent crystallization of sugar and retains flavors [3].

### 1.1.1 Extrusion

To incorporate fermented BSG as a food ingredient, extrusion method was used in this project. Extrusion is a method to convert raw food ingredients into food, like breakfast cereals, using thermal energy and pressure. The ingredients are mechanically forced by a screw through a pipe and a small opening which generates shear stress and high pressure. At the same time, the food ingredients are heated in different stages or zones where different temperatures can be applied. The shear, pressure, and heat change the properties in the ingredients and different structures and forms of the food can be obtained when it leaves the extruder. The food is normally referred to as extrudates when it leaves the extruder [4]. The extrudates can either expand directly after leaving the extruder, called direct expansion extrusion-cooking or after a second treatment like heating in convection oven or microwave, called delayed expansion extrusion-cooking [5].

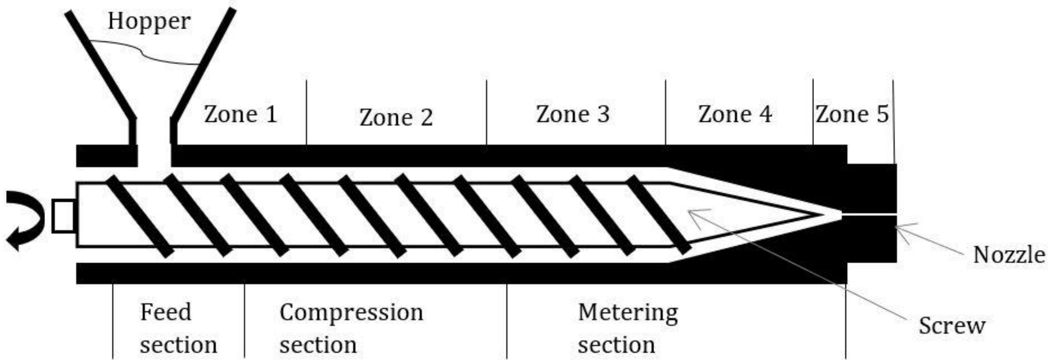


*Figure 1: A schematic figure of an extruder.*

Extrusion of foods containing BSG has been done in earlier studies, but not with bioprocessed BSG. When BSG was incorporated in ready-to-eat snacks the products were less expanded compared to products without BSG and the expansion was proportional to the amount of added BSG (10-30%). More BSG resulted in less expanded products. The phenomena are correlated to the high fiber content of BSG. The amount of starch in the extrudates was also highly positively correlated with the expansion and the texture of the extruded food products. The hardness of extruded ready-to-eat snacks was increased with a higher content of starch and more starch resulted in bigger radial expansion [6][7].

The water content of the food ingredients has a big impact on the extrusion outcome. A normal water content of the dough for extrusion of breakfast cereals is around 20% [8].

### 1.1.2 Texture

Different methods can be used to measure textures like hardness and crunchiness of breakfast cereals. This can be done either sensorially or mechanically. Mechanical tests do not provide all the information that a sensory panel can, but it is a comparable, cheaper and more convenient way to study texture. Various mechanical tests can be done such as penetration, three-point-bend, and compression [9]. In this project a penetration test was used.

### 1.1.3 Costumer panel – Focus Group

Focus groups can be used during food product development to get input from consumers about what kind of products they prefer. In a focus group several people meet to discuss the products and to identify their preferences. It is a good way to interview and gain input from several consumers at once.

## 1.2 Aim

One of the goals of the FUNBREW project is to find bioprocessing technologies for synthesis of functional compounds, like exopolysaccharides (EPS) and antioxidants, and to understand the biochemical and structural modifications occurring during biotransformation with the aim to use BSG as a food ingredient in cereal-based foods. The main aim of the overall project is to utilize fermented BSG as a novel food ingredient generating nutritional and economic benefits.

The goal of this master thesis project was to use extrusion to make a breakfast cereal containing around 30% fermented BSG, with a pleasant taste and texture. This master thesis project aimed to get an understanding of the process of making breakfast cereals with fermented BSG and what in the process that affects the taste, texture, and microstructure of the product. A big part of the focus in this project has been on developing a process and recipes in order to produce breakfast cereals with BSG and using the extrusion method.

# 2 Methodology

Several different tests have been performed in order to find a good process and recipe for breakfast cereal prototypes with fermented BSG. The method used for production of the final breakfast cereal prototypes will be presented first. Then a section with process development will be presented, the test performed which led to the final method. A workflow is presented below with the production steps (pink), tests performed (green) and evaluation methods used (yellow). Most evaluations have been done on the prototypes.

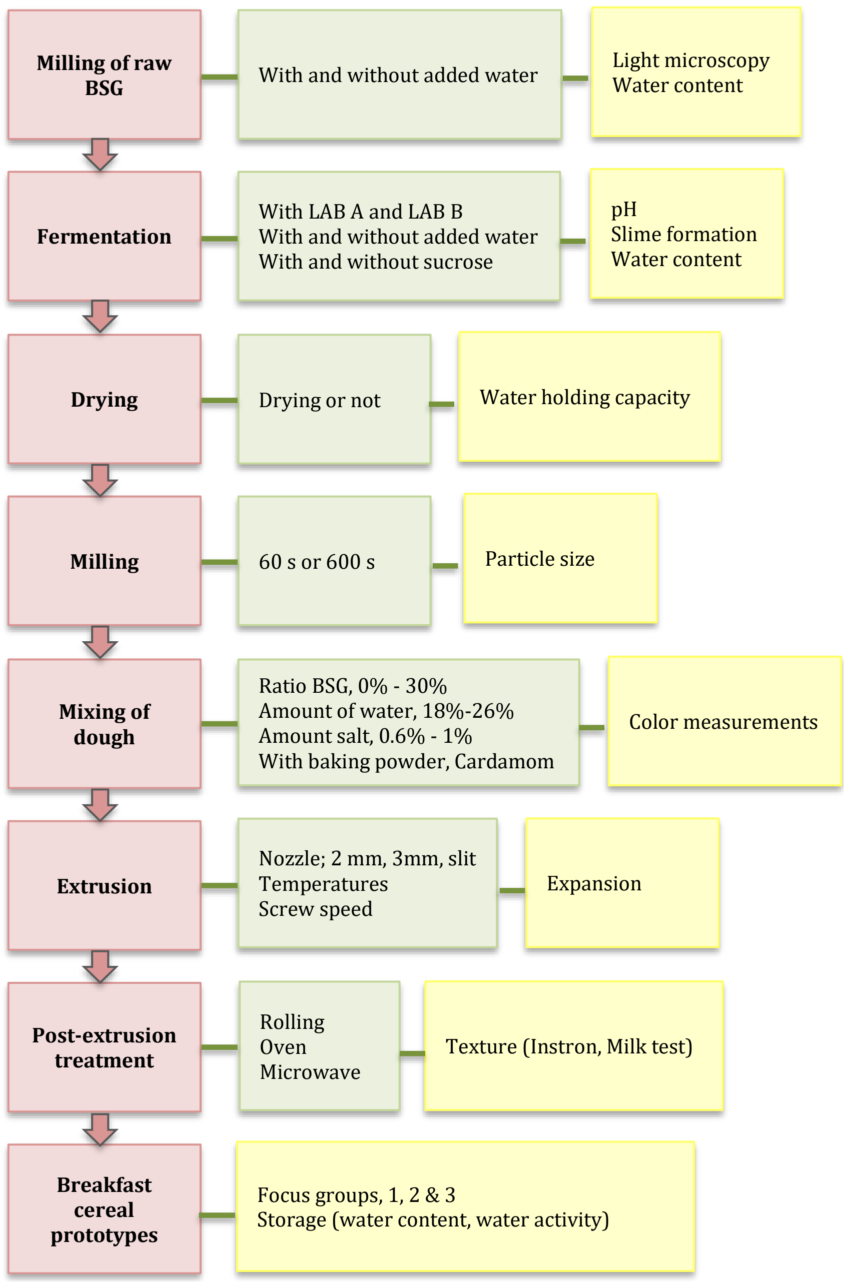


*Figure 2: Work flow*

## 2.1 Final process and recipe

### 2.1.1 Raw BSG

The BSG used in this study was mainly obtained from the brewery Dugges, a small local brewery in Gothenburg, but also the breweries Senson, located in Finland, and Peroni, located in Italy. The BSG, from Dugges and Senson, was stored frozen until tests were performed. BSG was slowly thawed at 5°C (±3°C) to prevent possible microorganism growth.

### 2.1.2 Milling of raw BSG

The BSG from Dugges was milled for six minutes in a Bosch kitchen blender before fermentation. BSG from Senson was wet-milled before arriving at RISE and BSG from Peroni was milled, bioprocessed (enzymatic treated and fermented with LAB) and dried before arriving at RISE by the group at University of Bari.

### 2.1.3 Fermentation

Fermentation was done with LAB, *Weissella confusa A16* (A) and *Leuconstoc pseudomesenteroides 20193* (B). These LAB species were the same as were used by the group at the University of Helsinki, but different from the LAB used at the University of Bari. The LAB used by the University of Bari was *Lactobacillus plantarum* PU1 and *Lactobacillus brevis* H4. *Leuconstoc pseudomesenteroides 20193* came from cane juice and *Weissella confusa A16* from African grains [1].

Bacterial growth maintenance was done by plating LAB A and LAB B on new MRS agar plates every week and incubating them at 30°C for 24-48 hours and then moving them to the fridge until next week. Bacteria were grown on plates instead of broth to confirm that it was a pure culture and not a mix of different bacteria species. To further confirm that it was the right species the LAB were grown on MRS agar plates with 2% sucrose, to see the slime production. The LAB were also Gram-stained and analyzed in a light microscope.

The fermentation recipe and protocol were adapted from the group at the University of Helsinki.

*Table 1: Fermentation recipe*

| | BSG (g) | Sucrose (g) | LAB (µl) |
|---|---|---|---|
| **With added sucrose** | 96 | 4 | 300 |
| **Without added sucrose** | 100 | - | 300 |

LAB were obtained from a culture grown overnight in General Edible Medium (GEM). Target inoculum was $10^6$ cfu/g of fermentation dough.

The fermentation recipe was upscaled to an amount of 2000 g total dough for the final fermentation.

#### 2.1.3.1 *Fermentation protocol:*

- Milled BSG, (water) and sucrose were added in a clean beaker.

- LAB were cleaned by sterile transfer of LAB from a culture grown overnight in GEM broth, to Eppendorf tubes. The tubes were centrifuged for 10 minutes, 10 000 rpm at room temperature. The supernatant was discarded.
- The pellet was resuspended in 1 mL physiological NaCl and centrifuged for 10 minutes, 10 000 rpm at room temperature.
- The supernatant was discarded, and the pellet was resuspended in 300 µl water and added to the BSG dough.
- The dough was mixed well, and the pH was measured.
- The sample was covered with parafilm and incubated at 25°C for 24 hours.
- pH was measured after incubation.

General Edible Medium (GEM) was prepared in 0.01 mol/L Potassium-phosphate buffer, pH 6.3, and sterilized by autoclaving. Ingredients mixed for 1000 mL media were 20 g glucose, 20 g sucrose, 43 g yeast extract and 1 g $MgSO_4 \times 7H_2O$.

Potassium-phosphate buffer was prepared by mixing 0.449 g $K_2HPO_4$ (molar weight: 174.18 g/mol) and 1.01 g $KH_2PO_4$ (molar weight: 136.086 g/mol) with distilled water until a volume of 1 liter [10].

For fermentation of BSG from Senson was water added before fermentation to reach a total water content of 83%, to be comparable with BSG from Dugges since raw BSG from Senson had a lower water content than BSG from Dugges.

To confirm the growth of LAB during fermentation, the pH was checked before and after fermentation. pH was lower after fermentation (from approximately pH 6.2 to pH 4.0) which indicated that the LAB had grown and produced lactic acid. Also, the lower pH prevented the growth of harmful bacteria.

### 2.1.4 Drying

The BSG was dried in a convection oven at 40-45°C after fermentation since temperatures above 60 °C can generate unpleasant flavors [11].

### 2.1.5 Milling of fermented BSG

After fermentation and drying of the BSG it was milled into a powder in a Bosch kitchen blender for 600 seconds to minimize the particle size of the BSG powder.

### 2.1.6 Mixing of extrusion dough

The final extrusion dough recipe used was: 30% fermented BSG, 59.2% rye flour, 10% potato starch and 0.8% salt (dry weight) and 26% total water content pre-extrusion. The dry ingredients were mixed with water shortly (approx. 15 min) before extrusion. It was noted that over time, the dough felt and behaved as though it contained less water, possibly an effect of hydration over time. Evaporation can be ruled out, since all materials were stored in sealed plastic bags.

### 2.1.7 Extrusion

All extrusion tests were performed with single screw extrusion and with a regular screw (without kneading elements). A slit nozzle (2 mm x 20 mm) was used to produce final prototypes. The extrudates were cut into short pieces, approximately 1.5 cm, right after exiting the nozzle, with scissors.

The extruder used had five temperature zones. The final process temperatures in the five zones used were 30/80/90/110/95°C. 50 rpm was chosen as the final process screw speed for slit nozzle.

#### 2.1.8 Post-extrusion treatment

The extrudates were expanded by microwave heating. 14 pieces were placed in a circle near the edge of the turntable and microwaved for 45 s, 750 W, Whirlpool, with revolving turntable.

#### 2.1.9 Final focus group - Third meeting

The samples tried and discussed during the third focus group meeting by the participants (7 persons, three males, four females) were the final prototypes, all with 30% fermented BSG, 59.2% rye flour, 10% potato starch and 0.8% salt (dry weight) and 26% total water content pre-extrusion. BSG used was dried at 40°C and milled for 600 s after fermentation. The samples were extruded with slit nozzle, screw speed 50 rpm, temperature 30/80/90/110/95°C, delayed expansion extrusion-cooking, expanded by microwave heating. Microwaved for 45 seconds, on a revolving turntable, 14 pieces per batch, pieces located in a circle near the edge of the turntable. Prototypes were:

- BSG from Dugges, fermented with sucrose
- BSG from Dugges, fermented without sucrose
- BSG from Senson, fermented with sucrose
- BSG from Peroni, bioprocessed (enzymatic treated and fermented at the University of Bari)
- BSG from Dugges, fermented with sucrose and 1.1% added cardamom

The participants first tried the breakfast cereals pure without stimuli, and then with stimuli (their own choice of milk, yogurt or "filmjölk"). Discussion topics of this focus group were; which of the prototypes that were their favorite and what characterized it, what was positive and negative with the prototypes and if they would have them as breakfast cereals.

All the participants regularly consumed breakfast cereals, daily or almost daily.

### 2.2 Process and recipe development

#### 2.2.1 Milling of raw BSG

The BSG was milled before fermentation. In the early stage of this project it was milled with added water, according to the protocol obtained from the group at the University of Helsinki; 1.8 kg BSG from Dugges was milled with 0.5 kg tap water. In later stages of the project, the milling was done without added water to minimize the water content in the material since it was not possible to extrude a dough with a too high water content, and to optimize the process by removing a step and minimize the volumes. The BSG from Dugges was milled for six minutes in a Bosch kitchen blender, and the time was obtained after a visual comparison of particle size with BSG from Senson.

### 2.2.2 Fermentation

Some tests were performed to compare fermentation with and without added water and with and without added sucrose (Table 2). The reason for comparing water addition was the same as to mill BSG without addition of water; to minimize the water content in the material since it was not possible to extrude a dough with a too high water content, and to optimize the process by removing a step and minimize the volumes during fermentation. The comparison with or without addition of sucrose aimed to compare how the slime, formed by the LAB when grown with sucrose, affected the extrusion properties and the prototypes: taste and texture.

*Table 2: Fermentation recipe*

| | BSG (g) | Sucrose (g) | Water (g) | LAB (µl) |
|---|---|---|---|---|
| **With water, with sucrose** | 36 | 4 | 60 | 300 |
| **With water, without sucrose** | 40 | - | 60 | 300 |
| **Without water, with sucrose** | 96 | 4 | - | 300 |
| **Without water, without sucrose** | 100 | - | - | 300 |

With or without water means extra added water excluding the water raw BSG contained pre fermentation. The added water was tap water.

Fermentation was performed as for the final prototypes, with the same LAB and protocol (section 2.1.2).

Antibiotics during growth or fermentation with LAB was not an alternative since the fermented BSG was used as a food ingredient.

Extrusion of non-dried fermented BSG was performed within 48 hours after fermentation for food safety reasons. In a later stage of the project the BSG was dried in a convection oven at 40–45°C after fermentation. The drying of fermented BSG made it possible to conserve it for a longer period.

#### 2.2.2.1 *Water holding capacity*

Measuring of water holding capacity (WHC) was done on dried BSG, fermented or unfermented, and the method was adapted from Miedzianka et al [12].

0.1 g of BSG was mixed with 10 g of distilled water and shaken in shake incubator for 1 hour. Samples were then centrifuged at 4000 g for 25 min, and the supernatant was discarded. The pellet was dried in an oven at 50°C for 25 minutes and samples were weighed. WHC was calculated according to equation:

$$WHC = \frac{B - A}{A} 100$$

Where A was the initial weight of the sample and B was the final dried weight of the remaining pellet.

#### 2.2.2.2 *Light microscopy*

The microstructure of BSG was characterized by staining of starch and proteins, and visualized by light microscopy (LM). Cell walls were visible without staining. LM was used to check the homogeneity of the milling and to analyze the microstructure after fermentation of BSG, and to compare the microstructure of BSG from Dugges and Senson.

The samples were freeze-sectioned with a cryostat, to slices with a thickness of 7µm, before LM was used. The samples were quickly frozen with liquid nitrogen to prevent any water crystals to form in the samples before slicing.

The samples were stained with Lugol and Light Green staining. Lugol stained the starch purple, while light green stained the proteins green. The cell walls remained unstained. Lugol consists of 5% iodine, 10% potassium iodide. Light Green consists of triphenylmethane.

### 2.2.3 Milling of fermented BSG

After fermentation and drying of the BSG it was milled into a powder in a Bosch kitchen blender. In an early stage of the project it was milled for 60 seconds. In a later stage of the project and the final process it was milled for 600 seconds to minimize the particle size of the BSG powder.

#### 2.2.3.1 *Particle size measurements*

The particle size of dried BSG was measured, and a comparison between milling for 60 s and 600 s was done. 50 g of each sample was used, and sieves used were 1.25 mm, 710 µm, 500 µm and 250 µm. Sieves were shaken at an amplitude of 1.5 mm, an interval time 10 s and a sieving time 10 min. The number of particles was measured by weighting particles at each sieve.

The effect of particle size on texture and taste of the product was evaluated by letting five, non-trained, participants blind taste the prototypes and give their opinion.

### 2.2.4 Extrusion

Variables altered during extrusion were nozzle size and shape, temperatures and screw speed.

Trials with three different nozzles were done, the nozzles used were 2- and 3-mm round nozzle and slit nozzle (2 x 20 mm). The extrudates were cut into short pieces, approximately 1.5 cm, right after exit of nozzle, with scissors.

Different extrusion temperatures were tested during the project in order to find a suitable process. In an early stage of the project, higher temperatures were used in order to get direct-expansion of the extrudates, initial temperatures used for slit nozzle were 30/90/130/155/170°C. Temperatures were then lowered in the last two zones, due to burning; 30/90/130/150/150°C. Extrudates were still direct expanding at this lowered temperature. In the later stages of the project were the temperatures lowered to temperatures were the extrudates were not direct expanded, like in the final process where the temperatures were 30/80/90/110/95°C in the five zones.

Several screw speeds were tried, and different screw speeds were used depending on the nozzle used. Lower screw speed (30-60 rpm) was used for round nozzle since the material exit the extruder faster for these nozzles, and higher screw speed (50-80 rpm) was used for slit nozzle. This parameter was not optimized for the process.

In the early stage of the project the extrudates were direct expanded, while at the end of the project were the extrudates expanded after a second treatment, either microwave heated, or convection oven heated.

#### 2.2.5 Extrusion recipe

The recipe was adjusted several times during the project depending on results from previous tests. Parameters that were evaluated were different BSG contents, water contents, salt contents, BSG from different breweries and addition of other ingredients.

*Table 3: Extrusion recipe variables tested*

| | |
|---|---|
| **Water content** | 18–26% |
| **BSG content** | 0–30% (dry weight) |
| **Salt content** | 0.6–1% (dry weight) |
| **BSG from different breweries** | Dugges, Senson, Peroni |
| **Other ingredients** | Baking powder, cardamom |

Rye was chosen as a suitable cereal raw material to combine with fermented BSG. The choice was based on results from the first focus group. The reference material used was a dough based on rye flour. Rye flour used was *Lantmännen fint rågmjöl* and potato starch used was *Garant potatismjöl*. Potato starch was added to increase the expansion of the extrudates.

The extrusion process was optimized on a recipe containing dried fermented BSG from Dugges brewery, fermented with sucrose, without added water and with LAB B. The optimization was done before different BSG sources and treatments were compared.

Evaluation of what ratio of fermented BSG that was most favorable for the process, texture and flavor were done. Dough recipes with 0, 5, 10, 15, 20, 25 and 30% dry weight fermented BSG were tested. Prototypes without BSG were used as reference, containing 89.2% rye flour, 10% potato starch and 0.8% salt (dry weight). The second focus group evaluated samples containing 15% and 30% fermented BSG. Prototypes with 30% unfermented BSG was used as control prototypes.

Dough with different water content were tested; 18, 23, 25, 26 and 30%. It was noticed that a higher fiber content required higher water content for extrusion. Water content used was 26% in 30% BSG samples while 23% water content was used for rye reference samples in the final process.

The salt content was adjusted two times during the project, from 1 g to 0.6 g to 0.8 g.

A test was done with the addition of cardamom, 1.1%, dry weight, to the dough before extrusion, in order to make a more flavorful breakfast cereal. This was done to a recipe containing 30% fermented BSG. Similar commercial products contain 0.7-1.8% cardamom [13][14].

Another test was performed with the addition of baking powder (sodium bicarbonate) to the extrusion recipe, one rye reference recipe and one 30% fermented BSG recipe, to investigate if it could lead to improved expansion and a crispier structure. Potassium bicarbonate is an ingredient in Quaker's Rågfras [16].

#### 2.2.5.1 *Color measurements*

The colors of the prototypes, with or without addition of baking powder, were measured with DigiEye by arranging the prototypes in heap under the camera, five replicates per sample. Colors were obtained in CIELAB color space, where *a* is the green-red scale and a higher value mean redder and a lower value greener.

### 2.2.6 Post-extrusion treatments

#### 2.2.6.1 *Rolling*

Tests to reduce the thickness and hardness of the extrudates were done by rolling extrudates with an Imperia pasta machine at setting 2 (2 mm). The extrudates were rolled after cutting into 1.5 cm long pieces with scissors.

#### 2.2.6.2 *Oven expansion*

Extrusion temperatures were adjusted to prevent material from burning and getting stuck in the nozzle: when using high temperature for direct expansion of extrudates, the material tended to adhere to the nozzle and get stuck. Therefore, the extrusion temperatures were lowered, and expansion was done with post-extrusion treatments. Tests with both convection oven expansion and microwave expansion were done and the results of the two methods were compared to each other.

For oven-expansion the extrudates were spread on a sheet and toasted in a convection oven for 10 minutes at 165°C (±5°C).

In an early stage of the project convection oven was used to toast extrudates, to obtain a crispier texture and a pleasant taste, for 10 minutes at 165°C (±5°C).

#### 2.2.6.3 *Microwave expansion*

A test was performed where 10, 15 or 20 pieces of *30% fermented BSG* extrudates were microwaved for 45 seconds, pieces placed in the middle of the turntable.

Another test was performed were 10 rye reference pieces were placed either in the center, covering an area of approximately 7 $cm^2$ of the turntable, or in a circle near the edge of the turntable. Test was performed due to notated within-batch variations when the pieces were placed in a pile in the middle of the turntable.

The final microwave settings used in the process were 14 pieces, 45 seconds per batch for 30% fermented BSG samples, and 10 pieces, 45 seconds per batch for rye reference samples, both placed in a circle near the edge of the turntable. The average weight per batch was 13.5 g for 30% BSG extrudates and 14.2 g for rye reference extrudates.

The expansion of extrudates was measured with a caliper by measuring the thickness of the extrudates.

#### 2.2.6.4 *Solubility in milk*

The solubility in milk was tested for several prototypes during the project in order to evaluate how the texture of the breakfast cereals changed with time in milk. Samples were placed in milk (3% fat, Arla, 12.5°C) and tested every fifth minute for 30 minutes by three, non-trained, participants. The texture and taste were sensory analysed and commented on.

### 2.2.7 Storage

All prototypes were stored in plastic bags in a constant room with humidity 14°C and relative humidity 30%.

Water activity was determined with an Aqualab 4TE water activity meter on prototypes after extrusion to ensure storability properties.

#### 2.2.7.1 *Water content*

The water content was measured by oven-drying, at 70°C for 24 hours under vacuum and in the presence of a water absorbent, and the weight compared before and after drying. This was done for several samples during the project, like raw BSG from Dugges and Senson, fermented BSG with and without added water, and extruded prototypes.

### 2.2.8 Focus group

Results from a focus group, performed at RISE by Lisa-Maria Oberrauter, during three different stages of the developing process was used. Some of the participants participated in all three focus group meetings, some at two of the meetings and some participants were new at each focus group meeting.

#### 2.2.8.1 *First meeting*

During the first focus group, the participants (six persons, two males, four females) discussed their preferences regarding commercial breakfast cereals. They tired and discussed the following breakfast cereals: Havre flakes original (AXA), Havre krisp blueberry (AXA), Frukost hjärtan with strawberries (ICA), Cornflakes (ICA), Bran flakes (ICA), Råg fras (Quaker), Havre fras (Quaker). All the participants were breakfast cereal consumers, 80% of them were consuming it daily or almost daily. The participants got questions about their consumption patterns, like how often they consume cereals, what kind of cereals and why they choose them. They were discussing the seven different kinds of commercial breakfast cereals, regarding if they would consume any of the cereals, what they would eat them with and if they perceived them as healthy.

#### 2.2.8.2 *Second meeting*

During the second focus group meeting the participants (eight persons, three males, five females) tried and discussed early prototypes with no BSG (rye reference), 15% fermented BSG, 30% fermented BSG or 30% non-fermented BSG. The samples were extruded with a 3 mm round nozzle and the temperature profile

30/80/125/130/130°C. Extrudates were direct-expanded, cut with scissors as it exited the nozzle into 1 cm long pieces, spread on a sheet and toasted in a convection oven for 10 minutes at 165°C (±5°C). Samples contained (% in dry weight):

- Rye reference: 89.4% rye flour, 10% potato starch, 0.6% salt
- 15% fermented BSG: 74.4% rye flour, 15% fermented BSG, 10% potato starch, 0.6% salt
- 30% fermented BSG: 59.4% rye flour, 30% fermented BSG, 10% potato starch, 0.6% salt
- 30% BSG: 59.4% rye flour, 30% BSG, 10% potato starch, 0.6% salt

All the participants were breakfast cereal consumers, and they were consuming it daily or almost daily.

The things the participants discussed were which of the four prototypes that were their favorite and what characterized it, what was positive and negative with the prototype and if they would have them as breakfast cereals. They were first trying the prototypes pure, and then in combination with their own choice of "filmjölk", yogurt or milk.

#### 2.2.8.3 *Texture measurements - Penetration test*

Penetration test was done with an Instron on the same kind of samples provided to the participants in the second focus group (rye reference, 15% fermented BSG, 30% fermented BSG and 30% BSG). A 2.5 mm probe was used and a penetration speed of 5 mm/s, 5 replicates were done for each sample. The maximum compressive stress and the mean modulus (hardness) was measured.

*Figure 3: Instron probe penetrating a rye reference sample.*

# 3 Results

The results are divided into two parts, first result from the process and recipe development will be presented. These results contributed to the final method used for production of the final prototypes. In the second part results from the final prototypes will be presented.

## 3.1 Process and recipe development results

### 3.1.1 Water content in raw BSG

Raw BSG from Dugges had a higher water content than BSG from Senson. BSG from Dugges located in the bottom of the barrel had a higher water content than BSG from the top of the barrel when the BSG arrived at RISE (Table 4). The mean water content of BSG from Dugges was 83%. Due to this result water was added, during fermentation of BSG from Senson, to a water content of 83% and BSG from Dugges from different levels from the barrel was pooled together before fermentation if possible. Water content batch variations occurred in the fermentation step during the project. How these between-batch variations affected the fermentation and the products was not fully evaluated, but no obvious differences were observed.

*Table 4: Mean (n=3) water content in raw BSG at three levels in the barrel.*

| Sample | Water (mean) |
|---|---|
| Dugges, top of the barrel | 81% |
| Dugges, middle of the barrel | 83% |
| Dugges, bottom of the barrel | 85% |
| Senson | 76% |

### 3.1.2 Milling with or without added water

No difference in particle size when milling BSG from Dugges with or without added water was observed by using LM. What could be observed was that BSG milled with added water was more diluted. Milling of BSG, in the later stage of the project, was done without the addition of water based on these results to make the process more efficient. It was easier to handle smaller volumes and less time was required to dry the fermented BSG when the water content was minimized.

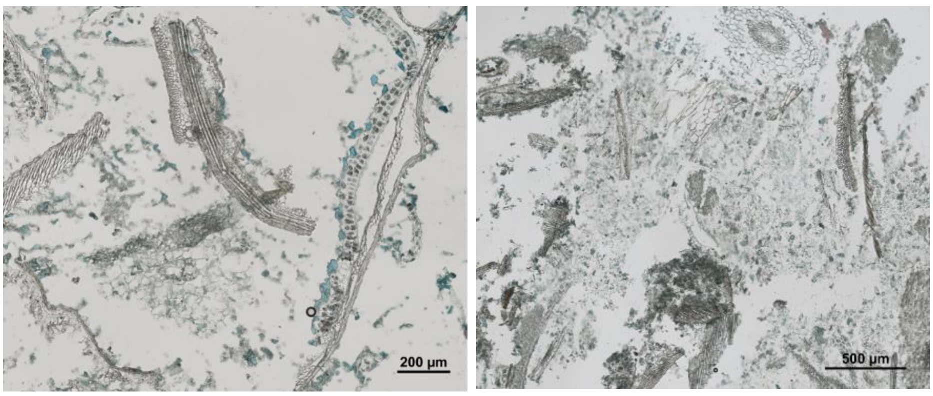


*Figure 4: Left: BSG from Dugges milled with added water. Right: BSG from Dugges milled without added water. Note: Different scales.*

LM observations showed that pre-milled BSG from Senson was more homogeneous with smaller particles than the milled BSG from Dugges which had bigger fragments of husks.

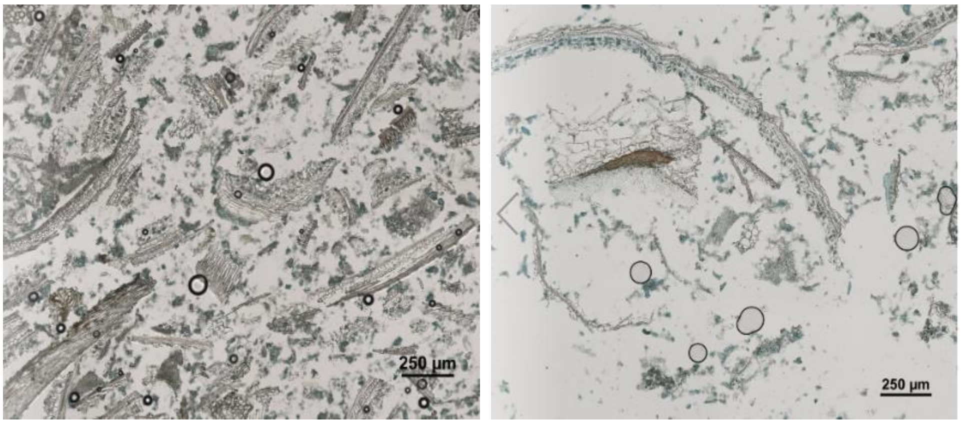


*Figure 5: Left: BSG from Senson. Right: BSG from Dugges.*

### 3.1.3 Fermentation with and without added water

Fermentation without added water generated a homogeneous result, where the slime was homogeneous spread in the dough, with noticeable slime production and a lowered pH. Fermentation with added water gave a separated fermentation dough where some BSG sank to the bottom of the beaker, and some of the BSG together with the slime floated on the surface of a water layer (Figure 6). pH was lowered in all three samples which indicates that the LAB had been growing (Table 5).

*Figure 6: Left: Added water before grinding and added water before fermentation (1). Middle: Added water before milling of BSG, but not before fermentation (2). Right: No added water (3).*

*Table 5: Left: Added water before grinding and added water before fermentation (1). Middle: Added water before milling of BSG, but not before fermentation (2). Right: No added water (3).*

| Sample | pH before fermentation | pH after fermentation | Difference in pH |
|---|---|---|---|
| **1** | 5.66 | 3.82 | 1.84 |
| **2** | 5.65 | 3.91 | 1.74 |
| **3** | 6.20 | 4.22 | 1.98 |

Fermentation was done without the addition of water in the later stages of the project due to this result. By eliminating the addition of water fewer steps were performed in the procedure, smaller volumes were handled and the drying of fermented BSG was faster.

The pH ranged from approximately 6.2 to 4.0 during fermentation for both LAB A and LAB B when fermenting without added water. Average pH for all fermentations performed during the project without added water was 4.0 and the fermented BSG had a distinct sour smell after fermentation.

### 3.1.4 Drying of BSG and Water holding capacity

When non-dried fermented BSG was used it was not possible to add BSG to a higher amount than approximately 4-5% dry weight due to its high-water content. The main reason to dry the BSG after fermentation was to be able to incorporate a higher amount of BSG into the dough without adding a too large amount of water which would affect the possibilities to extrude the dough. The effect of drying on the properties of the dextran slime was not evaluated.

What could be noticed when drying the fermented BSG was that the BSG with the highest amount of slime took a longer time to dry compared to BSG with less or without dextran slime. This was probably due to the slime's water holding capacity. When the water holding capacity was measured on the dried BSG, this effect was not noticeable, which could indicate that the fermented BSG with slime lost its water holding capacity when dried.

*Table 6: Water holding capacity mean value of four different batches of fermented BSG (with LAB B and sucrose) and two different batches of BSG.*

| Fermented BSG | BSG |
|---|---|
| 428 (std=22) | 508 (std=21) |

### 3.1.5 LAB slime production

During analysis of BSG in LM it was difficult to observe any differences in the microstructure between the fermented and non-fermented BSG from Dugges. Slime could not clearly be observed.

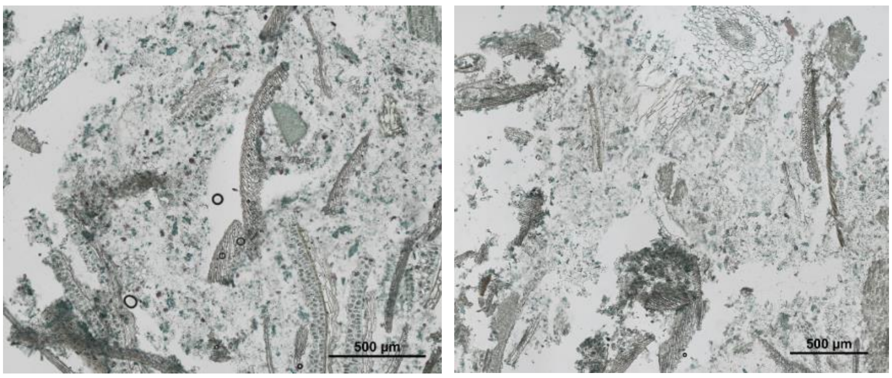


*Figure 7: Left: Fermented. Right: Unfermented.*

What could be observed was that LAB A produced more slime during fermentation than LAB B. Therefore, the extrusion process was optimized for a recipe with BSG fermented with LAB B and sucrose, since LAB B produce less slime than LAB A but more than none. By optimizing with a median amount of slime it was possible to apply the process on all samples without bigger complications, with either more slime or without slime.

### 3.1.6 Particle size

By milling the BSG for 600 seconds the particle size of the BSG clearly decreased (Figure 8 & 9). A small difference in texture and mouth feeling was observed by a few participants (n=5) that tried breakfast cereal prototypes extruded with either BSG milled for 60 seconds or 600 seconds. The participants described the prototypes extruded with the bigger particles as grassy and said that some particles got stuck in the mouth. They experienced the prototypes with smaller particles as crispier but a bit drier.

The particle size of the dried BSG powder did not clearly affect the expansion properties of the extrudates, but it did have a smaller effect on the moth feeling of the prototypes. Fibers in extrudates can disturb the starch network and inhibit the expansion. Smaller but more numerous fiber particles should disturb the network more, however, no effect on the expansion was observed.

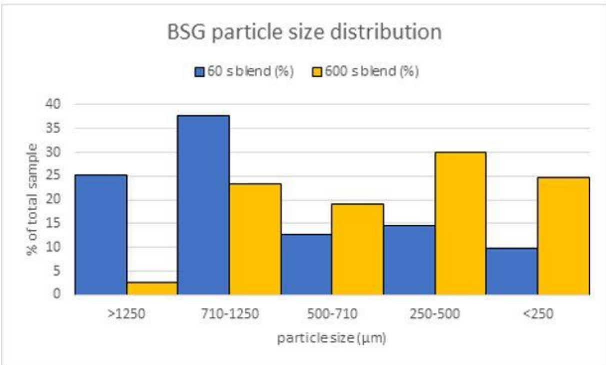


*Figure 8: BSG particle size distribution, 60 seconds milling and 600 seconds milling.*

*Figure 9: Left: BSG milled for 600 seconds. Right: BSG milled for 60 seconds.*

### 3.1.7 Extrusion

#### 3.1.7.1 *Nozzle*

The rationale for switching from 3 mm nozzle to 2 mm nozzle was to get a greater expansion due to the higher pressure a smaller nozzle would generate. A greater expansion was not observed when a 2 mm nozzle was used instead of a 3 mm nozzle.

The three different nozzles generated products with different kinds of appearance. Prototypes were easier to extrude with round nozzle when higher extrusion temperatures were used due to less burning, but the appearance of prototypes extruded with round nozzle was not liked by the participants in the second focus group. The prototypes extruded with the slit nozzle appeared more similar to commercial breakfast cereals and were preferred by the participants in the third focus group. A lower extrusion temperature and expansion in the microwave instead of direct expansion when coming out of the extruder made it possible to use a slit nozzle which gave a more pleasant looking breakfast cereal.

#### 3.1.7.2 *Shark skinning*

A recurring problem during the extrusion was shark skinning, a rough and gray surface appearing on the extrudates when exiting the nozzle. In most cases, the shark skinned extrudates barely expanded or did not expand at all. What caused the sharkskin of the extrudates was not fully understood, but what was done to avoid it was to lower the extrusion temperature, increase the water content of the dough slightly and adding water to the dry ingredients just before extrusion. Lowering the temperature in zone 4 and 5 had the largest impact on the reduction of shark skinning but did not eliminate the problem. With the final process method shark skinned extrudates were rare (temperature 30/80/90/110/95°C and water content 26% for samples with *30% fermented BSG*). Shark skinning was a pulsing and not a constant problem. Sometimes the extrudates got shark skin for a few seconds or minutes, while other times, not at all although nothing in the process was changed. This problem is something that needs further research when it comes to food extrudates. Some earlier studies have been done in this field on materials like plastic [17].

*Figure 10: Left: Extreme shark skin. Middle-left: Non expanded microwaved shark skinned BSG prototypes. Middle-right: Extrudates exiting extruder within some minutes, same batch of 30% fermented BSG, some pieces a little bit shark skinned, some not. Right: Material exiting the extruder a little bit shark skinned.*

### 3.1.8 Extrusion recipe

#### 3.1.8.1 *Extrusion with different amount of water*

Feed rate improved with the addition of more water. The feeding and outflow were faster with 23% water content in the extrusion dough than with 18% water content in extrusion dough.

Different materials behaved differently, and it was hard to find a standardized process. Different amounts of water had to be used for rye reference and samples with BSG. When a higher water content (>23%) was used for rye reference the outflow was higher in the middle of the nozzle than on the edges and the extrudate got a shape that was not preferred (Figure 11).

*Figure 11: Rye reference extrudate with a water content >23% pre-extrusion.*

### 3.1.8.2 *Salt content*

The early prototypes contained 1% salt which gave a too salty breakfast cereal prototype. The prototypes provided to the second focus group, contained 0.6% salt. In the second focus group the participants suggested a bit higher amount of salt and the amount was changed to 0.8% salt for the later prototypes, provided to the third focus group, and for the final products. 0.8% salt increased the flavor of the breakfast cereal prototypes without making them taste too salty.

### 3.1.8.3 *Baking powder*

When adding baking powder ($NaHCO_3$) the prototypes got a slightly green color, which did not look appetizing, and they got a metallic taste. Any difference in texture between samples with or without baking powder was not observed and baking powder did not improve the expansion of the extrudates. Baking powder was therefore excluded from further tests.

*Table 7: Colour measurement of Rye reference and rye with baking powder prototypes, were a higher a value means redder and a lower value greener on a red-green scale in the colour spectrum.*

| **Sample** | **a** |
|---|---|
| **Rye reference** | 7.4 |
| **Rye with baking powder** | 4.2 |

*Figure 12: Left: Rye reference prototypes. Right: Rye with baking powder prototypes.*

### 3.1.9 Post-extrusion treatments

Prototypes expanded less when BSG was added than in rye reference samples. Expansion decreased with the increasing addition of BSG, probably due to the fibers which inhibit the expansion [18] (Table 8).

*Table 8: Thickness of prototypes, mean value, n=10.*

| 0% fermented BSG | 15% fermented BSG | 30% fermented BSG |
|---|---|---|
| 11.7 (std = 1.1) | 10.1 (std = 0.9) | 7.0 (std = 1.0) |

*Figure 13: Left: 0% fermented BSG. Right: 30% fermented BSG.*

#### 3.1.9.1 *Rolling*

The difference in thickness, between rolled and unrolled breakfast cereal prototypes, were smaller the more BSG the samples contained. The difference in hardness for the samples, rolled or unrolled, with 30% BSG was very small due to the small difference in thickness. No obvious benefit of rolling was obtained and therefore rolling was excluded from the final process.

*Table 9: Thickness of rolled and un-rolled prototypes.*

| | 10% fermented BSG | | 20% fermented BSG | | 30% fermented BSG | |
|---|---|---|---|---|---|---|
| | **Rolled** | **Un-rolled** | **Rolled** | **Un-rolled** | **Rolled** | **Un-rolled** |
| **Average thickness [mm] (n=10)** | 3.07 | 3.38 | 2.31 | 2.58 | 2.04 | 2.18 |

#### 3.1.9.2 *Oven toasting*

When comparing direct-expanded extrudates with extrudates expanded in a convection oven, both rye reference samples extruded with 3 mm round nozzle, was it obvious that direct expansion generated a greater expansion. Average thickness (n=10) of the extrudates was for direct expanded: 6.03 mm diameter, and for oven expanded: 3.38 mm diameter.

The texture of oven expanded prototypes was harder compared to microwaved and direct expanded prototypes, but the prototypes got a pleasanter color by oven expanding, a more brown-golden instead of gray-brown color, and a smoother surface (Figure 14). The oven expansion method was abandoned due to too hard texture.

*Figure 14: Left: Oven expanded. Right: Microwave expanded.*

#### 3.1.9.3 *Microwave expansion*

When microwaving rye reference extrudates, 10 pieces, for 15 s, the expansion was incomplete; some large cells surrounded by non-expanded material. When microwaving 10 pieces for 30 s were the extrudates still not fully expanded. By microwaving the extrudates, 10 pieces, for 45 s, was the expansion good: small cells were evenly distributed, and the texture was crispy. When increasing the number of pieces to 20 for rye reference extrudates, the expansion was incomplete again, and the conclusion was that more material requires a longer time. When extrudates with 30% fermented BSG were microwaved, 10 pieces for 45 s, the extrudates were overdone and had a burned taste. The number of pieces was increased to 14 pieces, still 45 s, which generated good expansion for BSG samples. It is the weight per batch and not the number of pieces that is the crucial factor. With bigger (heavier) or smaller (lighter) pieces the number of extrudates per batch must be corrected. The average weight per batch was 13.5 g for 30% BSG extrudates and 14.2 g for rye reference extrudates.

The result of the experiment investigating the placement of the extrudates in the microwave, was that pieces placed in a circle at the edge of the turntable had less within batch and within piece variations than pieces placed in the center of the turntable. In the final process the extrudates were placed in a circle near the edge of the turntable.

It was possible to microwave extrudates more than 180 minutes after extrusion and with good expansion for both rye reference and fermented BSG extrudates. No difference in result was observed compared to extrudates microwaved directly after extrusion.

### 3.1.10 Focus groups

#### 3.1.10.1 *First meeting*

Most participants preferred Råg fras (Quaker), but a few preferred Havre fras (Quaker). The participants preferred these kinds of breakfast cereals because of the crispy and crunchy texture and because the cereals keep the texture without getting soggy to fast when mixed with milk, yogurt or “filmjölk”. They also preferred the taste of these breakfast cereals. Due to the high fiber content of Råg fras the breakfast cereals appeared healthy which made the participants feel good while consuming them. The participants said that they wanted crunchy and crispy cereals that were compact with a certain chewing resistance. The participants preferred rye-based cereals instead of oat-based cereals because they were richer in flavor and felt healthier. The participants preferred non-sweet cereals. Taste and texture were more important for the participants, when choosing breakfast cereals, than health aspects.

### 3.1.10.2 *Second meeting*

The participants said that the prototypes were different from commercial breakfast cereals, especial the appearance. They disliked the shape of prototypes. The participants said that all the prototypes had a neutral scent, but that sample *30% fermented BSG* had the strongest scent compared to the other samples. The scent reminded one of the participants of summer on the countryside with farmers having hay barns and some of the participants could feel the scent of raw cereals which they referred to as positive thing and said that it felt natural.

The participants thought that sample *30% fermented BSG* was richest in taste and tasted like sourdough crispbread, rye and cereals. They liked that the prototypes were not too sweet like commercial breakfast cereals and said that the sweetness came after a while of chewing. All the prototypes, according to the participants, had a neutral and boring taste and sample *30% fermented BSG* had a bitter aftertaste. Sample *30% fermented BSG* had the best texture followed by sample *30% BSG* since the participants felt that these samples had a more porous and crunchy texture that was easier to masticate, but they also thought that all the prototypes had too hard texture. Sample *30% fermented BSG* was the most preferred prototype since it had the best crunchiness, richest scent and taste according to the participants. Most of the participants would not buy or eat these prototypes due to their visual appearance. The participants suggested that a different shape and a bit more salt would improve the product.

The participants chose to mix the prototypes with either milk, yogurt or "filmjölk" based on their usual eating habits and preferences. Sample *30% fermented BSG* was still their favourite in combination with milk, yogurt or "filmjölk" due to texture and taste. The participants said they liked that the breakfast cereal prototypes did not get soggy when mixed with milk, yoghurt or "filmjölk".

*Figure 15: From left to right: 30% unfermented BSG, 15% fermented BSG, rye reference, 30% fermented BSG.*

### 3.1.10.3 *Texture measurements*

The results from the Instron measurements confirm how the participants in the second focus group experienced the breakfast cereals prototypes when it came to texture and hardness. The participants experienced the rye reference prototypes as hardest and both the modulus and the maximum compressive stress were highest for rye reference prototypes when measured with Instron. The 15% fermented BSG prototypes were next hardest and 30% fermented BSG and 30% unfermented BSG prototypes were least hard according to the participants which correlate well with the results from the Instron measurements.

*Table 10: Mean modulus and maximum compressive stress for samples provided to the second focus group.*

| Samples to second focus group | Modulus (mean n=5) [MPa] | Maximum Compressive stress (mean n=5) [MPa] |
|---|---|---|
| **Rye reference** | 218.3 | 11.3 |
| **15% fermented BSG** | 156.5 | 8.9 |
| **30% fermented BSG** | 119.2 | 7.5 |
| **30% BSG** | 113.9 | 7.3 |

When prototypes were placed in milk, during solubility tests, they keep their texture and crunchiness for a very long time (more than 20 minutes) which was something the participants in the second focus group experienced and liked.

#### 3.1.10.4 *Water content and water activity*

Water content was determined on the prototypes provided for the second focus group. This was done to ensure storability properties and food safety. The data obtained shows that the risk for microbial growth was low since both the water content and the water activity was low (Table 11 & 12).

*Table 11: Water content of products provided for the second focus group.*

| Product | Mean | Standard deviation |
|---|---|---|
| Rye reference | 5.4 | 0.56 |
| 15% fermented BSG | 4.8 | 0.34 |
| 30% fermented BSG | 3.2 | 0.47 |
| 30% BSG | 3.1 | 0.25 |

*Table 12: Water activity of products provided for the second focus group.*

| Product | Mean | Standard deviation |
|---|---|---|
| Rye reference | 0.3327 | 0.0155 |
| 15% fermented BSG | 0.2987 | 0.0062 |
| 30% fermented BSG | 0.2451 | 0.0017 |
| 30% BSG | 0.1945 | 0.0019 |

## 3.2 Final prototype results

### 3.2.1 Focus group - Third meeting

During the third focus group, the participants tried the prototypes:

- BSG from Dugges, fermented with sucrose
- BSG from Dugges, fermented without sucrose
- BSG from Senson, fermented with sucrose
- BSG from Peroni, bioprocessed (enzymatic treated and fermented at the University of Bari)
- BSG from Dugges, fermented with sucrose and 1.1% added cardamom

*Figure 16: Prototypes provided to the participants. From left to right: Dugges fermented with sucrose, Senson fermented with sucrose, Dugges fermented without sucrose, Peroni bioprocessed, Dugges fermented with sucrose and added cardamom.*

The participants said that the prototypes were a bit different from the breakfast cereals they normally consume, mostly based on the texture. According to the participants, the prototypes were more compact and less airy than the ones they normally consume, but this was a positive thing for the participants since they wanted a breakfast cereal which kept the shape and did not fall apart in the package. When the prototypes were tried pure, four of the participants had sample *BSG from Dugges, fermented with sucrose* as their favorite, two of the participants had sample *BSG from Peroni, bioprocessed* as their favorite, and one person had sample *BSG from Senson, fermented with sucrose* as their favorite and three participants also had sample *BSG from Dugges, fermented with sucrose and 1.1% added cardamom* as a favorite.

After trying with milk, yogurt or "filmjölk" were sample *BSG from Dugges, fermented with sucrose* favorite among the participants, but they also liked sample *BSG from Dugges, fermented with sucrose and 1.1% added cardamom. BSG from Dugges, fermented with sucrose* was the most preferred prototype mainly because of the taste, but also because of the texture and color. The participants liked the last prototype because of the cardamom which made the breakfast cereals tastier according to the participants. Something that was very important for the participants was that the breakfast cereals kept their crispiness in combination with dairy products after a while which the participants experienced that these prototypes did.

Some participants even preferred the prototypes *BSG from Dugges, fermented with sucrose* over commercial breakfast cereals. The participants liked this breakfast cereal prototype because they had an appealing taste: rye flavor, moderate and natural sweetness, round and full flavor, it was crunchy, did not stick into mouth/teeth, had the best color: looked well baked, and the prototypes felt healthy without having to much bran.

The conclusion after the third focus group was that sample *BSG from Dugges, fermented with sucrose* was the most preferred prototype and the final product for this master thesis project.

# 4 Discussion

### 4.1.1 Process and recipe application

As hoped for, the process and recipe developed made it possible to produce breakfast cereal prototypes with 30% fermented BSG with a pleasant taste and texture. It was possible to produce breakfast cereal prototypes with BSG from different breweries, BSG fermented with different bacteria and with and without slime producing LAB. The most preferred breakfast cereal prototype was the one the process was developed for, with BSG from Dugges and fermented with sucrose, which might have contributed to that this was the favorite. To produce a breakfast cereal prototype with BSG from another brewery or fermented without sucrose, the process and recipe can be adapted a little bit to enhance the taste of the products.

### 4.1.2 Raw BSG, sucrose and dextran content

The weight of BSG fermented with sucrose was bigger than the weight of BSG fermented without sucrose after drying even though they had the same weight pre drying. This extra weight could be due to sucrose remaining in the dough or dextran produced by the LAB using the sucrose. The amount sucrose added to the dough pre fermentation was based on the wet weight BSG and not dry weight which means that a bigger amount material dry weight was added, in the form of sucrose, to the dough than what was correlated for in the fermentation protocol since that was based on a wet weight of BSG, and wet BSG contains approximately 80% water. This means that the amount of raw BSG that was added to the extrusion dough in form of dried fermented BSG powder was a bit lower since the raw BSG in this powder was diluted in sucrose or dextran, compared to the BSG powder where the BSG had been fermented without sucrose. The extrusion dough with 30% fermented BSG, fermented with sucrose, contained approximately 24% raw dried BSG and 6% sucrose or dextran. Negative control with 24% fermented BSG, without sucrose, and 6% added sucrose or dextran to the extrusion dough, could have been used to compare with the 30% fermented BSG, fermented with sucrose, to confirm the necessity of adding sucrose pre fermentation. The same could have been done with 24% nonfermented BSG and 6% addition of sucrose or dextran to confirm the necessity of fermentation of BSG to obtain a tasty breakfast cereal.

Fermentation with sucrose clearly affected the taste of the breakfast cereal prototypes, and the prototypes fermented with sucrose was preferred. What could be discussed is if it was because the breakfast cereals still contained some sucrose or if the participants experienced the dextran as tasty, or if something else was happening when fermenting with sucrose which was beneficial for the taste of the prototypes. It could also be due to the fact that these prototypes contained a bit less raw BSG compared to the unfermented and fermented without sucrose prototypes (approximately 24% instead of 30%) since the raw BSG during fermentation gets a bit diluted in sucrose or dextran (or both, depending on if the LAB transformed all sucrose into dextran or not).

Contradictory to the theory that the prototypes with BSG fermented with sucrose were the most preferred prototype since the raw BSG was diluted and the prototype contained less BSG, is that the participants in the second focus group preferred the prototypes with more BSG, 30% fermented BSG instead of 15% fermented BSG. But on the other hand, a higher BSG content in these prototypes also means a higher sucrose or dextran content since the BSG was fermented with sucrose. However, the participants in the second focus group preferred the fermented BSG samples over the unfermented BSG sample which could be due to the sucrose or dextran.

### 4.1.3 Extrusion feeding rate

During some extrusion trials, it was easier to extrude samples with BSG fermented with sucrose than BSG samples fermented without sucrose since the later had slow throughput and/or tended to form shark skin. This was not fully evaluated, and no conclusion could be drawn since different phenomena caused the struggle when changing to samples with BSG fermented without sucrose. The slime might positively influence the extrusion properties, or the BSGs microstructure might be positively affected by the fermentation with the addition of sucrose.

The feeding of BSG fermented without sucrose was slower than feeding for samples with BSG fermented without sucrose during several extrusion tests. Samples with BSG fermented with sucrose were in most tests run before samples with BSG fermented without sucrose and the time could have been a factor influencing the feeding rate properties. The dough was mixed maximum 15 minutes before extrusion for these trials, but the extrusion properties might have changed in some way with time. However, the slower feeding rate probably was due to the feeding material content and might have been due to lack of slime in these samples.

### 4.1.4 Health and environment

The fiber content in the final breakfast cereal prototypes (30% fermented BSG) was approximately 25-31%. The amount added by BSG was approximately 15-21%, and the amount added by the rye flour approximately 9%. Recommended daily intake of fibers for an adult according to Svenska Livsmedelsverket is 25-35 g, which means that 100 g of this kind of breakfast cereals cover the daily fiber intake for an adult person [19]. Similar products like Råg fras (Quaker) contains 13% fibers [16]. Protein content in the cereals in the final product was approximately 11-14% since BSG contains 20-30% protein and rye flour contain 8.5% protein [15].

The final breakfast cereal prototypes had approximately the double amount of fibers compared to commercial high-fiber breakfast cereals and they were made of re-used food ingredients which makes them a good alternative as a breakfast cereal both in a health aspect and environmental aspect. To feel healthy when eating breakfast cereals was something the focus group participants said was important for them when choosing breakfast cereals, and they said that it was a plus if the breakfast cereals were good in an environmental aspect. Most people in Sweden consume less fibers than the recommendations from Livsmedelsverket, and new tasty high-fiber products are needed to help people increase their fiber consumption. To see commercial breakfast cereals with fermented BSG might be the future due to its benefits: good taste and texture, healthy and environmentally good alternative to commercial breakfast cereals.

### 4.1.5 BSG variations

BSG is a heterogenous material, and there are variations in the BSG depending on how it has been treated at the breweries but also where it comes from and how the barley has been treated during and after harvesting. These variations are due to the fact that BSG is a waste product from the main product for the breweries, and how the BSG is will depend on the breweries main product: beer. BSG can contain different kinds of grains which will differ depending on what kind of beer the breweries are producing and on their process. In this study the BSG from Dugges and Senson contained barley while the BSG from Peroni contained barley and some corn. The BSG from Senson was pre-milled at the brewery while the BSG from Dugges was not and had to be milled before the fermentation to be comparable. The process developed in this project can be applied on different kinds of BSG but might have to be adjusted depending on the structure, micro and macro, and content in the BSG, both water content and the grains used.

#### 4.1.6 Drying and up-scaling

By drying the fermented BSG it can be stored for a longer period which makes it possible to ferment bigger batches than the amount needed for one batch of products. This would make it easier to up-scale the process, but it is negative since it requires energy and applies an extra step in the process. On the other hand, this means that no consideration needs to be done regarding the variation in the water content of the BSG pre drying.

#### 4.1.7 Further studies

##### 4.1.7.1 *Fermentation benefits*

Something that was never compared was breakfast cereal prototypes with either non-fermented BSG or BSG fermented without sucrose. This would have been an interesting test in order to know if there were any difference and if the fermented prototype was preferred over the non-fermented. What is known from this study is that fermented with sucrose was preferred over both fermented without sucrose and non-fermented, but it is not known how they perform over each other. These results could give the answer whether it was the sucrose/dextran or the fermentation itself that contributed to the preference in taste by the participants. Fermentation with sucrose might stimulate production of tasty compounds, like ethanol or other secondary metabolites, which could influence the taste of the breakfast cereal products.

##### 4.1.7.2 *BSG as a novel food ingredient*

As could be observed by looking at the LM pictures of fermented and unfermented BSG, LAB is not good at degrading fiber structures. By using enzymes or other microorganisms like fungi, which better can degrade the structure, a release of nutrients like proteins might occur in the product. Alternatively, it might be possible to use that protein in other types of food. To extract vegetable proteins and extrude it into a vegetable protein source is trend right now and something that might be possible to do in future studies about BSG as a novel food ingredient.

# 5 Conclusions

The conclusion of this master thesis project was that it was possible to make breakfast cereal products with a content of 30% fermented BSG with extrusion method with a pleasant taste and texture.

An improved knowledge and understanding of what in the process of making breakfast cereals, with fermented BSG, that affects the taste, texture and microstructure of the products have been gained.

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