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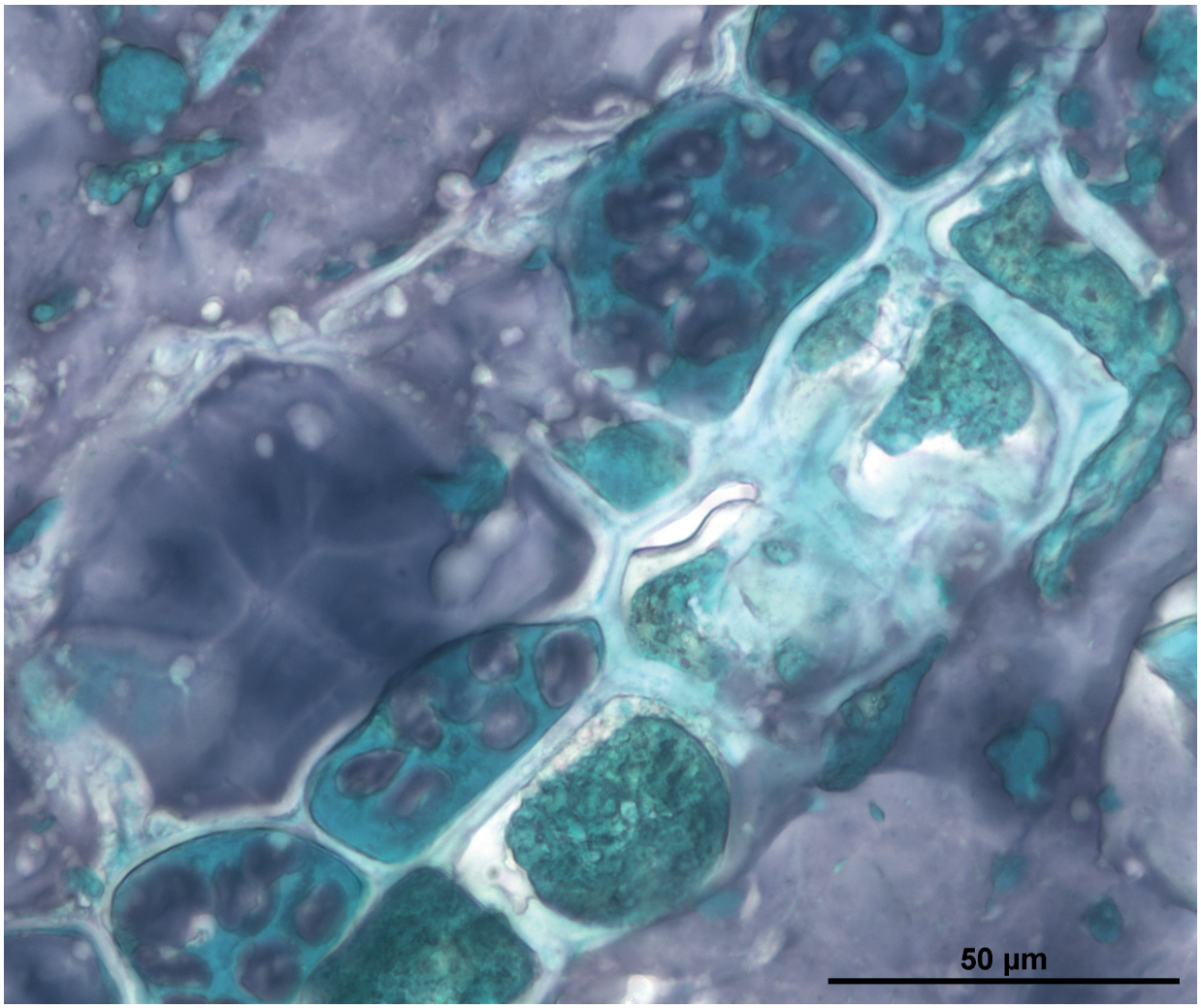

Characterization of extruded breakfast cereals containing Brewers' spent grain

Evaluation of its microstructure, textural and sensory profile compared to conventional cereals

Master's thesis in Biology and Biological Engineering

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Department of Biology and Biological Engineering
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Gothenburg, Sweden 2020

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The research was performed at
RISE – Research Institutes of Sweden, department of
Agrifood and Bioscience, Gothenburg, Sweden

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Examiner: Anna Ström, Department of Chemistry and Chemistry Engineering, Division of Applied Chemistry, Chalmers

Coverpage: Micrograph of beakfast cereal containing 30% bioprocessed BSG. Magnification 40x.

Master's Thesis 2020
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Svensk sammanfattning

Drav (BSG) är den största biprodukten vid ölframställning. Drav består till största delen av skaldelar från korn och är rikt på fibrer, protein samt fenolföreningar. Idag används dravet främst som djurfoder på grund utav dess fiberrika och heterogena sammansättning som medför begränsade processegenskaper.

Med hänseende till dess näringsvärden och höga tillgång året runt är det av intresse att kunna återanvända dravet inom livsmedelsindustrin. Att fermentera livsmedel för att öka biotillgängligheten av bioaktiva ämnen samt förbättra strukturen har ökat i popularitet på senare tid. Att använda BSG som substrat vid fermentering kan vara ett sätt att förbättra dess funktionella egenskaper vid användning i cerealiebaserad mat för människor. Polysackariden dextran som produceras *in situ* under fermenteringen med utvalda mjölksyrabakterier är känd för att förbättra textur och sensoriska egenskaper.

I detta master arbete fermenterades drav med *Leuconostoc pseudomesenteroides* och frukost flingor innehållandes 30% drav tillverkades genom extrudering. Mikrostrukturen på dessa flingor karaktäriserades med konfokalmikroskop och ljusmikroskop. Textur och sensoriska egenskaper utvärderades instrumentellt och med hjälp utav en tränad panel. Prototypflingorna undersöktes både torra och efter uppblötning i mjölk, samt jämfördes med konventionella flingor.

Den mikrostrukturella analysen visade en kompaktare och mindre porös struktur till följd av förekomsten av stora fibrer som tros ha en armerande effect. Detta påvisades även i den texturella analysen med hårdare struktur och bättre bibehållen krispighet efter uppblötningen i mjölk jämfört med konventionella flingor. PCA analys av den sensoriska utvärderingen visade att dravet medför egenskaper som hårdhet, krispighet och mörk färg. Undersökningen av bioprocessen med mjölksyrabakterier kunde visa på ett fragmenterat proteinnätverk. Cellväggar innehållandes AX sågs nedbrutna vilket indikerar ökad biotillgänglighet av fenoliska substanser bundna till lignocellulosiska strukturer i cellväggarna.

Nyckelord: drav, *Leuconostoc pseudomesenteroides*, extrudering, mikrostruktur, konfokalmikroskop, ljusmikroskop, biotillgänglighet, textur analys, sensorisk analys, PCA

Abstract

Brewer's spent grain (BSG) is the major by-product in the beer-brewing process. BSG consists mainly of the grain husks of barley and is rich in fibre, protein and phenolic compounds. The main application of BSG to date is limited to animal feed due to its fibre-rich and heterogenous composition giving it poor technological properties.

Because of its nutritional composition and high availability throughout the year BSG is of interest for subsequent use in the food industry. Use of fermentation for baked food products to promote the bioaccessibility of the bioactive compounds and improve the structure has increased in popularity lately. To use BSG as a substrate for fermentation with selected LAB may be a way to improve its functional properties for incorporation in cereal based food for human consumption. The exopolysaccharide dextran that is produced *in situ* during fermentation with selected LAB is known to improve textural and sensory attributes.

In this master's thesis study, BSG was fermented with *Leuconostoc pseudomesenteroides* and breakfast cereals containing 30% bioprocessed BSG (w/w) was produced with extrusion cooking. The microstructure of those breakfast cereal prototypes was characterized with confocal laser scanning microscopy (CLSM) and light microscopy (LM). The texture and sensory attributes of the products were evaluated both instrumentally and sensorially with a trained panel. The prototypes were assessed both as dry and after immersion in milk and compared to conventional breakfast cereals on the market.

Microstructural analysis of the prototypes visualized a more compact and less porous structure due to the presence of larger fibres assumed to have a reinforcing effect. This was further proved in the textural analysis with harder textures and better retained crispiness after soaking in milk compared to conventional cereals.

PCA analysis of the sensory evaluation revealed that BSG render attributes like hardness, crispiness and dark colour. The evaluation of the bioprocessing with LAB revealed a more fragmentized protein network and AX were seen released from the cell walls, implying increased bioavailability of phenolic substances bound to lignocellulosic structures in the cell walls.

Keywords: BSG, *Leuconostoc pseudomesenteroides,* extrusion, microstructure, CLSM, LM, bioavailability, textural analysis, sensory analysis, PCA

List of abbreviations

BSG: Brewer's spent grain
CLSM: Confocal laser scanning microscopy
LM: Light microscopy
EPS: Exopolysaccharide
LAB: Lactic acid bacteria
AX: Arabinoxylan
NA: Numerical aperture
TPA: Texture profile analysis
RATA: Rate-all-that-apply
GEM: General edible medium
CFU: Colony-forming unit
BSA: Bovine serum albumine
PCA: Principal component analysis
RISE: Research institutes of Sweden

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Last, I would thank my examiner Anna Ström for taking the time to review my thesis and giving me feedback.

Table of content

1. INTRODUCTION

This master's thesis was part of the FUNBREW research project that aims at developing novel functional food products with recycled brewer's spent grain from the brewing industry.

The FUNBREW project is comprised of a collaboration of four institutions: University of Helsinki (Finland), University of Bari (Italy), University of Bozen-Bolzano (Italy) and RISE Research Institutes of Sweden. This master's thesis project was performed at the division of Product Design and Perception at RISE Agrifood and Bioscience in Sweden.

1.1 Background

Brewers' spent grain (BSG) is the solid residue of barley malt remaining in the beer brewing process as a by-product (Figure 1). Per every 100 L brewed beer there is approximately 20kg BSG produced and the annual global production is estimated to 39 million tonnes which makes it the primary by-product of the brewing industry (Lynch, Steffen, & Arendt, 2016a, 2016b).

In previous studies BSG has been incorporated in bakery products like bread and ready-to-eat snacks (Lynch *et al*., 2016b). Incorporation of BSG at levels above 10% has been reported to negatively alter the textural and sensorial attributes of the final product (Mussatto *et al*., 2006). Findings in the literature reports enhancements of nutritional and technological properties of BSG when pre-treated with xylanase followed by fermentation with selected LAB (Ktenioudaki *et al*., 2015). To the best of my knowledge, no previous study has been focused on incorporation of 30% (w/w) bioprocessed BSG in breakfast cereals to obtain a functional food product, which was the scope of this present work.

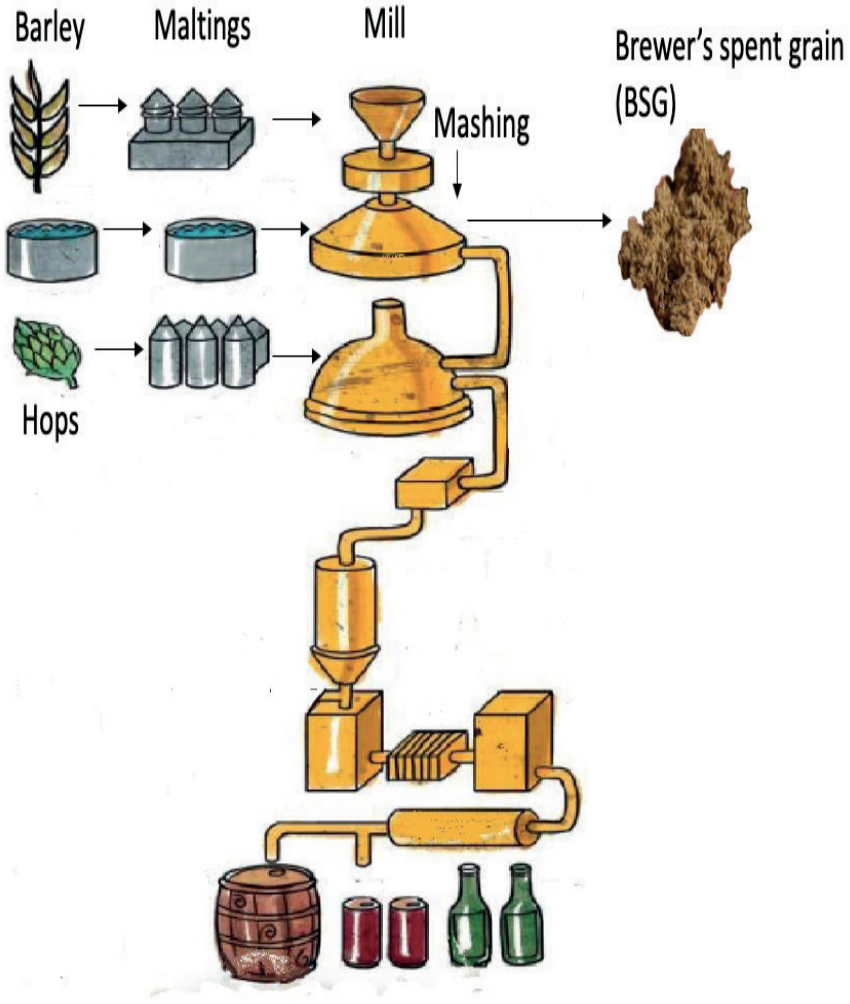


Figure 1. Beer brewing process from Barley to bottles via BSG.

1.2 Barley

BSG is essentially composed of the grain husk of the barley left after the wort production. The barley grain consists of three main parts: germ (embryo), endosperm (aleurone and starchy endosperm) and grain coverings. Figure 3 displays the barley kernel (Mussatto, Dragone, & Roberto, 2006).

The aleurone layer is found between the pericarp and the hyaline layer of the endosperm and is the outer layer of the endosperm. It can be either single-layered or multicellular divided by cell walls depending on cereal type.

The aleurone layer is involved in storage and protein bodies or aleurone grains are enclosed in the aleurone cells (Antonini et al., 2018). Barley has a multicellular aleurone layer while the aleurone layer in rye contains one row of cells (Figure 2). Comparisons between different prototypes in this study were focused on multicellular aleurone structures.

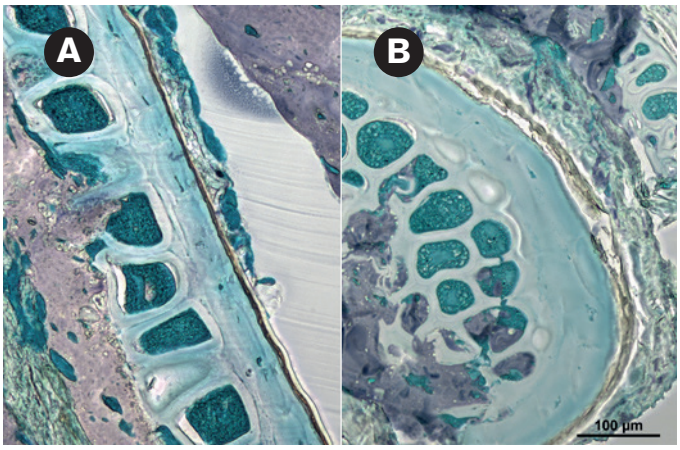


Figure 2. Aleurone structure from A) rye and B) BSG.

1.3 Structural characteristics and chemical composition of BSG

In addition to grain husk from the barley BSG contains insoluble proteins. Animal feed is as of now the main use for this product but because of its fibre and protein-rich composition, 30-50% and 19-30% respectively, and high availability throughout the year BSG has great potential for subsequent use in food industry (Lynch et al., 2016b; McCarthy, O'Callaghan, Piggott, FitzGerald, & O'Brien, 2013).

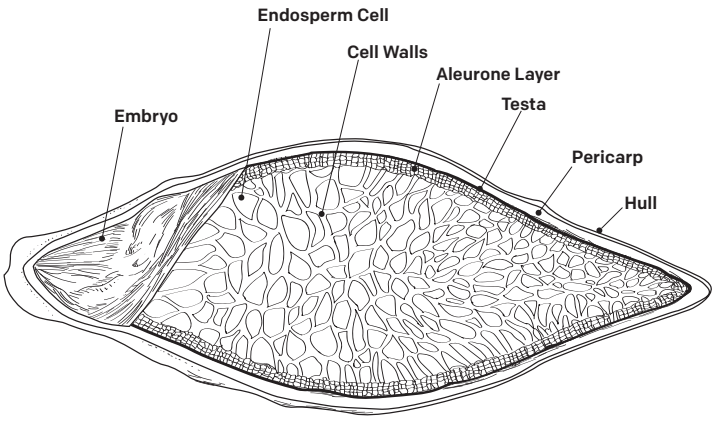


Figure 3. A longitudinal section of a barley kernel with inspiration from Mussatto et al., 2016

Moreover, components present in BSG such as ferulic acid and p-coumaric acid brings promising health benefits for human such as its potential to prevent against heart disease in which reactive oxygen species are involved (Salawu, Bester, & Duodu, 2014).

Ferulic acid in BSG is bound to the lignocellulosic structures of BSG and has low bioaccessibility. It is therefore desirable to improve the bioavailability of BSG through bioprocessing technologies (McCarthy et al., 2013). The main reason for its limited utilization is due to its high fibre content and complex structure that gives it poor technological properties (Title et al., 2020).

The chemical composition of BSG may vary depending on raw material, adjuncts added and the malting and mashing regime used in the brewing process (Lynch et al., 2016a). The husk-pericarp-seed coat layers that mainly constitute BSG contains cellulose, hemicellulose like arabinoxylan (AX), lignin, protein and fat. Vitamins like biotin, folic acid, choline, riboflavin and thiamine are found in BSG. Also, the presence of minerals like Ca, Cu, Fe, Mn, K and N have been reported (McCarthy et al., 2013). BSG is classified as a lignocellulosic and heterogenous material (Mussatto et al., 2006). Table 1 lists the approximate chemical composition of BSG.

Table 1. Composition of BSG (%).

Component	Percent dry weight (%)
Cellulose	12–25
Hemicellulose	20–25
Lignin	12–28
Total protein	19–30
Starch	2–3
Lipids	10
Ash	2–5

(Lynch et al., 2016b; Mussatto et al., 2006; Qin, Johansen, & Mussatto, 2018)

Starch

Starch is stored mostly in the endosperm in the form of granules whose content and arrangement depends on the type of cereal. Amylopectin and amylose constitute the starch granules in a 3:1 ratio approximately (Antonini et al., 2018). The granules can be classified into A- and B-type. The A-type granules are larger (15-40μm) and shaped like lentils whereas type-B are smaller (2-10μm) and spherical (Antonini et al., 2018).

Starch gelatinisation is the process when the crystals disintegrate to an amorphous structure. Below the gelatinization temperature this is a reversible process but above this temperature irreversible changes occur such as swelling of the starch granules and solubilization (Robin & Palzer, 2015). A higher degree of gelatinisation of the starch implies a more rapid digestion and also a more porous structure in the product (Poutanen, Flander, & Katina, 2009). Thus, the structure of the starch is interesting both from a nutritional point of view but also related to the texture of the final product.

However, the starch content in BSG is low due to the enzymatic degradation of the starchy endosperm and filtration of the wort (Lynch et al., 2016b). The breakfast cereals in this study contain 30% BSG and mostly rye flour (56-70% starch) hence, starch is an influential component in terms of consumer liking of the final product.

Arabinoxylan (AX)

AX is a hemicellulose included in the group "non starch polysaccharides" present in the cell walls of the plant. The cell wall composition varies between different plant species (Dornez et al., 2011). AX content in barley is between 3,4-8,0% (Collins et al., 2010).
From a nutritional point of view AX are correlated to beneficial health effects in human as it is classified as dietary fibre. Thus, indigestible by enzymes in the human GI-tract. Arabinoxylan chains is built up of β-(1,4)-linked xylose units. The arabinose over xylose ratio (A/X) describes the degree of substitution of xylose with arabinose residues, which is a parameter of importance for structural aspects. Phenolic compounds such as ferulic acids and p-coumaric acids also affect the structure of AX (Dornez et al., 2011).

1.4 Bioprocessing and hydrocolloids in food

Fermentation of cereal foods are drawing increasing attention due to its potential to bring about positive health benefits and improve sensory and textural attributes in fibre-rich foods. Modifications of the matrix due to fermentation may impose improved bioavailability of certain micronutrients such as minerals and phytochemicals (Poutanen et al., 2009). Solubilisation and hydrolysis of proteins and polysaccharides in the grains are examples of desirable physicochemical alterations. Moreover, production of functional metabolites like the exopolysaccharide dextran is an example of a desired effect of bioprocessing with selected lactic acid bacteria (LAB) (Kajala *et al*., 2016).

Hydrocolloids represent a group of hydrophilic polymers extensively used in food formulations as structuring agents to improve the rheology and texture of the product. The chains of the hydrocolloids can interact and hold water, acting as a thickener (Wang *et al*., 2018). Hydrocolloids can also impose similar characteristics as gluten in terms of viscoelasticity and gas binding properties to promote a more expanded and light texture of the final product. Improved gas retention is obtained by the ability of the hydrocolloid to stabilize the liquid film surrounding gas bubbles in the cereal dough to prevent them from collapsing (Wang *et al*., 2018). Furthermore, incorporation of hydrocolloids may also improve the processability in the extruder as the gelled network entails increased lubricity and altered viscosity of the dough within the extrusion barrel (Ozilgen & Bucak, 2018).

Adding dextran by *in situ* production by LAB during fermentation may also introduce other positive metabolic effects while still meeting the consumer demand for 'clean' labelled food without additives. To use dextran produced by the LAB-strain Leuconostoc for this purpose in bakery products is approved by the European Commission (CS/NF/DOS/7/ADD, 18/10/2000) (Verheugen, 2005).

Based on previous studies within this project the dextran producing LAB-strain of choice in this thesis work was Leuconostoc *pseudomesenteroides* DSM 20193.

During the fermentation process the LAB interact with components in the grain and produce lactic acid and acetic acid and thus, lowering the pH. The change in pH selectively activate amylases, proteases, hemicellulases and other enzymes that are present (Poutanen *et al*., 2009).

1.5 Prototypes and focus groups

In previous studies within this project a final recipe had been developed for a prototype containing 30% fermented BSG and 59,2% rye flour with input from consumer panels and focus group meetings. Based on this recipe with rye flour used as starch matrix eight different samples were made with BSG obtained from three different breweries: Dugges (Sweden), Senson (Finland) and Peroni (Italy) (Figure 4). For each BSG a bioprocessed sample was produced along with its corresponding non-bioprocessed controls. A rye flour sample without BSG was also included as a reference.

The cereal prototypes evaluated in this thesis work are presented in Table 2

Figure 4. Extruded breakfast cereal prototype containing 30% BSG (w/w).

***Table 2**. List of breakfast cereals samples.*

Abbreviation	Prototype
D.BSG	Dugges, non-fermented
D.B+	Dugges, fermented with sucrose
D.B-	Dugges, fermented without sucrose
S.BSG	Senson, non-fermented
S.B+	Senson, fermented with sucrose
P.BSG	Peroni, non-bioprocessed
P.Bio	Peroni, bioprocessed
Rye ref	Reference, rye flour

1.6 Extrusion cooking

Extrusion-cooking was the method of choice to produce breakfast cereals in this project. Extrusion-cooking is a method that enables the production of food with textures that are ready to eat (Robin & Palzer, 2015). A schematic diagram of the extruder is showed in Figure 5. The dough is forced through different temperature zones in the extruder where transformation of the food materials takes place under the influence of high shear and pressure (Anton & Luciano, 2007).

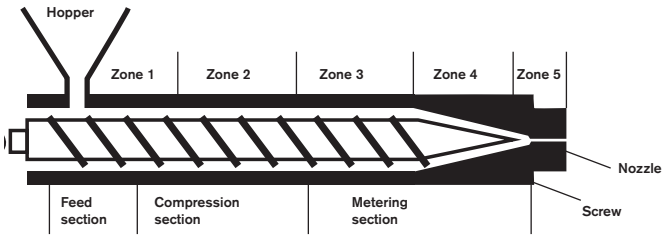


Figure 5. Extruder

1.7 Microstructure

Analysis of the microstructure of food is of great importance in food science. The microstructure influences the sensory attributes of the product such as texture, taste perception and appearance. Microscopy analysis can help unravel and understand the properties and behaviour of a food material (Autio & Salmenkallio-Marttila, 2001).

Furthermore, by using specific stains it is possible to localize and describe the organisation of certain nutrients in cross sections of cereals in order to access their bioavailability. To develop new cereal-based foods or change specific attributes of food, an overall understanding of the components' microstructures are essential (Jekle & Becker, 2015). The structure and components of the grain thus forms the basis for this. Microscopy is the technique applied for evaluation of the microstructure in this study.

1.7.1 Light Microscopy

Light microscope has a range that works for a variety of processed foods and is a useful tool to qualitatively visualize cereal grain structures (Autio & Salmenkallio-Marttila, 2001).

Visible light (λ 400-800nm) from a halogen lamp is used as light source and the area of interest on the specimen is uniformly illuminated. A set of lenses are used to enlarge the image and the collector lens concentrates the light to a certain area on the specimen examined. Different objective lenses are used to administer the magnification.

Resolution in the image is dependent on magnification and the numerical aperture (NA). Higher NA gives brighter images as the objective collects light more efficiently (Microscopy & Carlsson, n.d.).

1.7.2 Confocal laser scanning microscopy (CLSM)

A schematic diagram of the confocal system in CLSM is illustrated in Figure 6.

The principle behind the confocal system in CLSM is the detection of excited light from a point location in the specimen (Mauko, Muck, Mirtič, Mladenovič, & Kreft,

2009). An illumination pinhole limits the out-of-focus light hence, a limited object field is illuminated. This differs from light microscopy where a whole object is illuminated. Lasers that emit lights at certain wavelengths are used as light sources in CLSM and are projected on the specimen by the scanning mirrors. The sample are scanned pointwise by the laser and the detected light is converted to information that forms the basis for the image being built up by the system's software (Jekle & Becker, 2015). A 3D perception of the sample can be obtained with the corresponding software when stacks of images (z-stacks) are gradually gathered through an optical slice of the sample (Mauko et al., 2009).

An advantage of the CLSM over LM is the ability to study bulk samples with less sample preparation which reduces the risks of contamination and modifications of components being studied (Part C Food Structure Analysis, Characterisation, and Modelling, n.d.).

Aromatic substances like lignin can be visualized without staining due to their autofluorescence but for visualization of other components selective staining methods or labelling with fluorochromes are usually used (Jääskeläinen, Holopainen-Mantila, Tamminen, & Vuorinen, 2013).

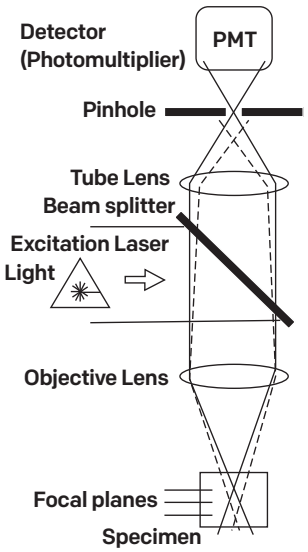


Figure 6. Schematic diagram of a confocal laser scanning microscope (CLSM).

1.7.3 Fluorescent dyes and immunolabelling

Fluorescent dyes forms either non-covalent or covalent bonds. For the latter, immunolabeling is the commonly used technique. The immunolabelling technique facilitate the precise localization of specific compounds *in situ*. This technique relies on the specific binding capacity of antibodies to antigens. Detectable labels are used, either on the primary antibody or on a secondary antibody, that enables visualization of the formed complex by CLSM or other microscopy techniques (Vázquez-Gutiérrez & Langton, 2015).

Direct and indirect antibody labelling differ in the number of antibodies being used. In direct labelling only a primary antibody specific for the antigen is used with a label attached. Whereas in indirect antibody labelling the label is bound to a secondary antibody directed against the primary one. The latter variant was used in this study as greater sensitivity can be obtained due to signal amplification (Scientific, n.d.).

1.7.4 Sample preparation for microscopy

Cross-sections of the breakfast cereals examined in this study were prepared by two different methods based on application and target. For examination with light microscope cryo- sectioning was the method of choice as it is a rapid method that enables larger sections that can be easily stained and visualized. This technique did not work for too dry samples as they crumbled and did not hold together. Hence, sampling for cry-sectioning was done right after extrusion cooking and before baking in the microwave oven. The samples to be sectioned is frozen in liquid nitrogen and mounted onto specific holders upon cutting. Then transferred onto specific glass slides before staining and microscopic analysis.

For samples intended for immunolabelling a more stable method was used: plastic-embedded sections. Thus, enabling examination of puffed samples taken after the micro-baking. Plastic embedding can facilitate the cutting of very thin sections either by a motorized microtome or other specialized knife equipment (John D. Bancroft, 2012). 1-4 μm sections were used in this thesis study; semi-thin sections.

1.8 Texture analysis

Alongside sensory properties like taste and appearance, texture is a fundamental characteristic of a product in terms of consumer liking. For breakfast cereals where puffed products with great expansion is preferred texture plays a key role. Crispiness and crunchiness are desired attributes opposed to sogginess (Robin & Palzer, 2015). What determines a products textural characteristics is its structure at various scales; supramolecular, micro and macro, which in turn are affected by the raw material and its modifications during processing. Also, the particle size of the flour plays a crucial role for the texture of breakfast cereals produced by extrusion cooking as a too wide size distribution correlates with an uneven water distribution (Robin & Palzer, 2015).

There are various methods to measure textural attributes like hardness and brittleness of food products. Penetration, compression and three-point-bend are tests that can be performed to assess textural parameters (Anton & Luciano, 2007). The texture profile analysis

(TPA) of extruded products like breakfast cereals by an instrument can never replace a sensory evaluation performed by a trained panel but it is a cheaper and less time consuming approach that can provide useful information about some rheological parameters and for comparative purposes (Valles Pamies, Roudaut, Dacremont, Le Meste, & Mitchell, 2000).

The force required to penetrate a sample measured with an Instron universal testing machine can be recorded in a force-deformation curve from which parameters such as compressive stress at maximum compressive load, Young's modulus and number of peaks can be determined (Anton & Luciano, 2007).

The maximum force, i.e. the stress at maximum compressive load, can be taken as a measurement for hardness of the product (Chanvrier et al., 2013). For evaluation of a solid material's stiffness Young's modulus (E) can be used. Young's modulus relates the elastic deformation of a material caused by load and is defined in Equation 1 (Roos, 1995). The lower the Young's modulus, the more compressible material.

$$\sigma = E\epsilon \qquad (1)$$

Where σ and ϵ refer to tensile stress and strain of the solid material.

Crispiness can be related to the total number of measured force peaks (Npeaks) along the jagged curve (Anton & Luciano, 2007). However, this is not a fully adequate method if it does not consider the shape and magnitudes of the peaks.

Textural attributes that were addressed within the scope of this study were: hardness, crispiness and stiffness. The method used was a penetration test with an Instron machine.

1.9 Sensory analysis

Quantitative comparisons between different products based on specific attributes can be obtained through sensory analysis performed by a panel of trained assessors; the sensory panel. Methods commonly used in food industry and research include the Texture Profile Method, Quantitative Descriptive Analysis® (QDA®) and Spectrum™ Descriptive Analysis. The time and cost required to recruit and train a group of panellists is a limiting factor for these approaches therefore more rapid methods that still maintains robustness have been developed (Fleming, Ziegler, & Hayes, 2015).

Rate-All-That-Apply (RATA) is an efficient method to provide sensory characteristics of a product. The basic principle of RATA is that the panel rates all attributes that apply to the product of interest with the use of point anchors correlating to intensities of the attributes (Traill, Luckman, Fisk, & Peng, 2019). In this study a five-point anchor was used rating the intensities from low to high. Since this is a rapid method it is understood to not yield the same detail level as that of descriptive analysis, but it is assumed to give sufficient information for characterization of a product's sensory profile and define similarities and differences among products (Traill et al., 2019).

The main goal of a sensory evaluation is usually to classify the products but the performance of each panellist and of the panel as a whole are important for the analysis too. Therefore, univariate analysis of each descriptor along with analysis methods that takes interaction effects into account are usually performed (Romano, 2007).

1.9.1 Data analysis

For the analysis and interpretation of sensory data various statistic functions can be used. Univariate models that estimate the variances or squared deviations connected to each factor are used to analyse attribute by attribute to assess the significance of differences between the products evaluated. The largest noise in the sensory data is assumed to be contributed to the panellist-product interaction and hence, it is important to take into account ("Basic Statistical Concepts for Sensory," 1986).
A mixed model two-way ANOVA including both product differences, panellist differences and panellist-by-product interactions was used in this master's thesis work (Equation 2). Only product differences are fixed while both panellist differences and interactions are considered random. Panellists and interaction effects are considered random because the panellists are treated as a random representative of a population (Romano, 2007).

$$X^k_{ijm} = \mu^k + \alpha^k_i + \beta^k_j + \alpha\beta^k_{ij} + e^k_{ijm}$$
$$e^k_{ijm} \sim N(0, \sigma^2) \qquad (2)$$

Where μ^k is the mean for attribute k. The main effects of the products are represented by α^k_i and β^k_j is the main effects for panellists. The panellist-product interaction term, $\alpha\beta^k_{ij}$, represent differences between panellists' in their measurements. The error term, e^k_{ijm}, is the residual variation as a result of replicates and is assumed to be uncorrelated and normally distributed with the same variance. This model assumes homogeneity of variance (Luciano & Næs, 2009).

1.10 Aim

A product's consumer acceptance and success are strongly related to its textural and sensory attributes. To be able to develop techniques to successfully recycle the BSG from breweries into functional breakfast cereals it is crucial to establish a fundamental understanding for the effects of BSG and bioprocessing methods on the final product.

The aim of this master's thesis work was to characterize the final breakfast cereal prototypes containing 30% (w/w) bioprocessed BSG from three different breweries: Dugges, Senson and Peroni and relate their microstructure to textural and sensorial characteristics. The intention was further to reveal the impact of bioprocessed BSG in extruded breakfast cereals and the influence of dextran. The prototypes were also compared to conventional products to establish an overall evaluation of the production process.

The hypothesis was that the transformation occurring to BSG under fermentation, caused by the LAB and the presence of dextran, would yield an improved structure and enhanced nutritional and sensorial attributes of the final product.

Limitations

Composition of the ingredients that make up the cereal dough and process parameters for the fermentation and extrusion process will not be evaluated within the scope of this project. Moreover, not all breakfast cereal samples were included in all different analyses performed.

2. MATERIALS AND METHODS

2.1 Fermentation and extrusion

2.1.1 Raw Material

The BSG used in this project was obtained from Dugges (Sweden), Senson (Finland) and Peroni (Italy).
The BSG from Peroni differed from the others as it was obtained pre-treated from beer brewed with barley malt and maize in a 70:30 ratio. This BSG was milled in a laboratory mill (Ika-Werke M20, Germany) and bioprocessed with both hydrolytic enzyme Depol™ 761P (Biocatalysts, USA) and LAB (*Lactobacillus plantarum* PU1 at 30°C for 24 h).

BSG from Senson was obtained wet-milled but un-treated and BSG from Dugges was obtained completely untreated and was both wet-milled and dry-milled at RISE. The LAB-strain used in this thesis study was *Leuconostoc pseudomesenteroides* 20193 (gift from University of Helsinki).

The proximate composition of the flours was determined in previous studies. Cereals were made based on a recipe made at an earlier stage (Table 3).

***Table 3**. Final recipe of one batch breakfast cereals prototypes containing 30% (f)BSG (dry weight).*

Component	Weight (g)
Rye flour	175,2
Potato starch	29,6
(f)BSG*	88,8
Salt	2,4
Water	85,8

*f=fermented BSG

2.1.2 Bacterial culturing

The fermentation process carried out was based on a protocol developed at an earlier stage of the project by researchers at the Department of Food and Nutrition Sciences, University of Helsinki (Verni *et al*., 2020). The LAB strain was cultivated on Man-Rogosa–Sharpe (MRS)-agar plates at 30°C for 24 hours. For preparation of the fermentation process, the strain was sub-cultured in General Edible Medium (GEM):

i. First, 0,01M Potassium phosphate buffer was prepared by combining 800ml milli-Q water with 0,449g K_2HPO_4 [0,0026M] and 1,01g KH_2PO_4 [0,0074M] and add milli-Q water to a final volume of 1000ml.
ii. GEM was then prepared by combining 20g glucose, 20g sucrose, 43g yeast extract, 0,874g $MgSO_4$ anhydride and 916g 0,01M Potassium phosphate buffer in a beaker. The medium was autoclaved.

2.1.3 Inoculating bacteria in BSG for fermentation

The frozen BSG was thawed in refrigerator for 72hr upon mixing. 1000g BSG per batch was mixed at full speed for 6 minutes (Bosch Multitalent3, with chopping blade). For the BSG from Senson's brewery this step could be omitted as the BSG was already wet milled. Water was added to the BSG from Senson to a total water content of 83% before fermentation to correspond to the water content of the BSG from Dugges. For *in situ* formation of dextran 4% (w/w) sucrose was added to the BSG (B+). For dextran-negative samples (B-) those were prepared with the same starters but without the sucrose.

Microbial cells were transferred from cultures incubated overnight in GEM through centrifugation (10 000g, 10 min), washed once with NaCl (phys.) and inoculated into the cereal dough at a target inoculum of 10^6 CFU g^{-1} dough. The doughs, both dextran-positive and dextran-negative, were covered with parafilm and fermented for 24 h at 25 °C. To ensure a successful fermentation process, pH was measured before and after fermentation (Table 4).

Table 4. pH before and after fermentation.

	pH	
	Before fermentation	After fermentation
D.B+	6.18	4.0
D.B-	6.18	4.24
S.B+	6.18	3.9
P.Bio*	5.79-5.95	3.87-3.97

*Samples obtained pre-treated from the University of Bari (Verni *et al*., 2020).

2.1.4 Drying and milling of BSG

After completed fermentation the BSG was dried in a convection oven at 40°C for 24h to remove excess H_2O and increase its storability.

For the non-fermented BSG, 1000g BSG per batch was mixed at full speed for 6 minutes (Bosch Multitalent3, with chopping blade) and dried in a convection oven at 40°C for 24h too.

The dried BSG (Dugges and Senson) was milled for 600s (Bosch Multitalent3, with chopping blade) at full speed to obtain a suitable particle size of the BSG flour. Cereal dough mixtures were prepared according to Table 3 with the water added just prior to extrusion.

2.1.5 Extrusion cooking

The cereal dough was extruded by single screw extrusion (Collin Teach Line 20T-E single screw extruder, Germany) using a slit nozzle (2 mm x 20 mm). The extruder had five temperature zones and the temperatures were set at 30, 80, 90, 110 and 195°C. A screw speed of 50 rpm was kept constant for all extrusions. The exiting ribbons were cut into pieces of approximately 1,5 cm with scissors and baked in a microwave for 45 seconds (Whirlpool "Talent", 750 W, with revolving turntable; grill function not used) 14 pieces per batch. Samples were thereafter placed in plastic bags and stored at 14 °C.

2.2 Sample preparation for microscopy

2.2.1 Resin embedding

Two different kinds of resin embedding-systems with two different procedures were carried out to compare the methods.

Pieces of breakfast cereals (approximately 5mm) were fixed with 37% formaldehyde stabilized with $CaCO_3$ and methanol together with some glutaraldehyde at room temperature for three days, dehydrated in a series of acetone solutions (Appendix A) and infiltrated with a Historesin Embedding Kit (Kulzer Technovit® 8100) for four days. All steps carried out at a constant temperature of 4°C (Leica EMTP, Leica microsystems GmBH, Austria) and in a fume hood.

For the second method (Kulzer Technovit® 7100) all steps were carried out in room temperature and in a fume hood. Two samples of each cereal samples (approximately 5mm) were put in four small beakers and submerged in a series of ethanol solutions (90, 96, 100%) for dehydration and infiltration according to a protocol (Appendix A).

2.2.2 Microtomy prior to immunolabelling (CLSM)

One cereal piece was embedded per sample block for both methods and cut into semithin sections (4 µm) with a PT-PC ultramicrotome (PowerTome, RMC products, USA). The plastic block was put in the sample holder which was put into the trimming block. The plastic block was thereafter trimmed with a razor blade in a trapezium shape with about 1mm plastic left around the sample. Sections cut were then transferred to a water drop on a Thermo Scientific Polysine adhesion slide with the aid of a forceps and allowed to dry into the glass slide in room temperature prior to immunolabelling

2.2.3 Cryo-sectioning prior to light microscopy (LM)

To section the frozen cereal samples the cryo-sectioning technique was used with a cryostat (Leica CM1900, Leica Microsystems Germany). The thickness of 10 µm was set to fit analyses with both Light microscope (LM) and confocal laser scanning microscope (CLSM). The temperature was set to -14°C for both the knife and the chamber.

The cereal samples were trimmed with a hand knife into smaller size (1/3 of a cereal; about 5mm) and mounted on the sample holder with Pelco® Cryo-Embedding compound (US) before submerged into liquid nitrogen for quick freezing. The samples were thereafter placed in the cryostat in order to reach the requested temperature of -14°C before sectioning. Slices were cut and put onto either Polysine blue slides (Thermofisher) or Superfrost microscope slides (VWRTM). The microscope slides were kept on heating block (2208 Multiplate, LKB) for 5-10 min to facilitate the attachment of the sections onto the slides.

2.2.4 Immunolabelling of Arabinoxylan

For immunolabelling of AX the indirect method with primary and secondary antibodies was used. The primary antibody LM11 AX (Plantprobes) and the secondary antibody Alexa Fluor™ 647 goat anti-rat IgG (H+L) (Invitrogen, ThermoFisher) were applied to semithin sections (4µm) on Polysine coated microscope slides (Thermo Scientific). LM11 AX binds to unsubstituted and low-substituted xylans and AX (McCartney, Marcus, & Knox, 2005). The protocol followed was an adaption from previous studies carried out by Dornez *et al*., All incubations were performed in a moisturized

chamber and the samples were then stored in a dark environment in a refrigerator.

i. Slides were fixated for 30 min in 4%formaldehyde in 0,01M PBS, pH 7,4.
ii. Rinsing three times with 0,01M PBS, pH 7,4, for 5min each time.
iii. Blocking was then done with 2,5% BSA in 0,01M PBS, pH 7,4 for 40 min.
iv. The primary antibody LM11 AX was diluted 1:50 in 0,5% BSA in 0,01M PBS, pH 7,4 upon incubation for 2,0hr. An applicable negative control was made by substituting the primary antibody for 0,5% BSA in 0,01M PBS, pH 7,4.
v. A rinsing procedure according to step ii was performed once again.
vi. Subsequently the fluorescently labelled secondary antibody was diluted 1:500 in 0,5% BSA in 0,01M PBS, pH 7,4 and then incubated for 2,0hr under aluminium foil to avoid exposure to light.
vii. The final rinsing procedure, step ii.
viii. One drop of ProLong™ Diamond Antifade Mountant was added to each slide and a cover slip was put on top of the sections to avoid fading of the fluorescent dye.
ix. Slides were sealed with nail polish.

2.3 Confocal laser scanning microscopy (CLSM)

2.3.1 Microstructure analysis of the cross section

The pore structures of the breakfast cereals were analysed with CLSM (Leica TCS SP 5, Germany) and a HCX PLFLUOTAR 5.0x0.15 dry objective, zoom 1x and 3x. Cross sections were stained with Texas Red® sulfonyl chloride (1mg/µl in H_2O) (Invitrogen, Thermo Fisher scientific) that primarily binds to proteins, but also stains other components in the sample.

A HeNe laser with λex = 594 nm and an Ar/ArKr laser with λex = 488 nm were used as light sources. Signals were captured at 500-580 nm and 605-700 nm for autofluorescence and Texas Red®. The image resolution was 1024 x 1024 pixels and a line average of 1 was used for all micrographs captured.

2.3.2 Microstructure analysis of the surface

For analysis of the breakfast cereals' surface microstructure CLSM was used. The same instrument and settings as for the cross-sectional analysis was used but the samples were visualized unstained as signals from autofluorescence were captured.

2.3.3 Visualization of Arabinoxylans (AX)

Analysis of the immunolabelled sections was carried out by CLSM with a HXC PL APO 20.0x0.70 immersion objective. Light sources used were a HeNe laser with λex = 594 nm and a HeNe laser with λex = 633 nm. Signals were captured at 500-570 nm and 660-710 nm, respectively. The resolution of the micrographs was 1024 x 1024 pixels and a line average of 4 was used for all micrographs. Red and green colours were assigned to visualize the antibodies as red and other material as green.

2.4 Light microscopy (LM)

The sections were observed using a Nikon microphot-FXA microscope and images were captured with a DFK33UX264 458 10252 camera and processed with the NIS-Elemens D software (Nikon Instruments Inc., USA).

Samples observed were taken both before and after baking in the microwave oven. All samples were taken after extrusion cooking. The objectives used were 10X, 20X and 40X.

2.4.1 Staining for light microscopy

Different stains were utilized to visualize different structures of the samples. Lugol's Iodine was used to visualize starch, LightGreen for the proteins and a mixture of Lugol's Iodine and LightGreen was used to obtain comprehensive micrographs of the overall structure.

2.5 Instrumental texture analysis

Ten different breakfast cereals, the eight prototypes studied in this project along with to commercial products: Råg Fras® (Quaker) and All-Bran® (Kellogg's) were instrumentally examined regarding their textural attributes.

Penetration test dry samples

A puncture test was carried out using an Instron 5542 universal testing machine (USA) equipped with a 500N load cell and fitted with a 2,4-mm diameter cylindrical flat-faced probe. The speed of advance was set to 10 mm/min. All samples' anvil height [mm] were measured prior to punctuation with an electronic calliper at the highest point of each sample. 10 replicates per sample were carried out.

Penetration test after immersion in milk (bowl-life)

The breakfast cereals were immersed in whole milk (3% fat per 100ml) for 10 min in a bowl (diameter 9.5 cm) and then allowed to quickly drain on paper towels prior to measuring. The analysis method was the same as for the texture analysis of the dry samples. 10 replicates per

sample was done for the bowl-life analysis and the milk volume was 60 ml.

2.6 Particle size evaluation

A comparison between particle size for the BSG obtained from the three different breweries (Dugges, Peroni and Senson) was carried out. 30-50g of sample was shifted for 10 min with an amplitude of 1.5 and sieving intervals of 10s in a Fritsch Analysette PR547 (Germany) using sieves with hole sizes of 1250, 710, 500 and 250µm. 2 balls were added per sieve to facilitate the sieving. After the sieving was stopped, the BSG retained on each sieve was weighted and the percentage was calculated from the total mass of sample added. Fractions passing through the 250µm sieve and collected at the bottom plate were considered 'fine' fractions whereas fraction larger than 500 µm were considered 'coarse' in this study. One replicate per sample was run.

2.7 Sensory analysis

The sensory characteristics of the breakfast cereal samples were evaluated by a trained panel in two replicates using the method Rate-All-That-Apply (RATA). For the sensory evaluation six of the breakfast cereal prototypes with BSG from two different breweries were tested. The samples tested are listed in Table 5 and visualized in Figure 7. The samples were stored in sealed plastic bags in 14°C prior to testing.

Table 5. Samples for the sensory evaluation.

Samples	
S.BSG	Senson unfermented
S.B+	Senson fermented with sucrose
D.BSG	Dugges unfermented
D.B+	Dugges fermented with sucrose
D.B-	Dugges fermented without sucrose
Rye ref	Rye reference

2.7.1 Panel and training

Nine trained panellists were recruited (n=9), initially selected according to the guidelines of ISO 8586:2014 all female and aged between 35 and >60 years old. One training session (3 h) was held prior to the evaluation. With inspiration from Cartier et al., (2006), a sorting exercise was done where the panellists were asked to group the samples according to similarities and differences; both flavour, appearance and texture. This was done to get the panellists familiarized with the product space of the breakfast cereal prototypes to be evaluated. During the training session commercial and well-known products were used as references to help differentiate and define the attributes. A final list of attributes and definitions was generated (in Swedish) comprising 23 attributes for the dry samples and 19 attributes for evaluation of bowl-life characteristics (Table 6).

Figure 7. Breakfast cereal prototypes evaluated in the sensory analysis. Upper section from left to right: D.B+, D.B- and Rye ref. Lower section from left to right: S.BSG, D.BSG and S.B+.

Table 6. List of sensory attributes, definitions and references for sensory evaluation of the breakfast cereal prototypes.

Category	Attribute	Definition
Appearance (D)	surface smoothness	degree to which the surface is even or smooth[1]
	dark colour	degree of darkness[2]
	expansion	cross-sectional expansion
Odour (D)	hay-like	hay-like smell
	roasted	heated oat flake (150 °C, 30 min)[3]
Texture (D+W)	hardness	force required to bite completely through[4]
	crispiness	brittleness and noise intensity of the product during the mastication[3]
	adhesiveness	how much the sample get stuck in the teeth[3]
	chewiness	chews needed before swallow[3]
	splinters/coarse mouthfeel	degree of coarseness in crumb in mouth[2]
	compactness	compactness of cross section of sample[1]
	homogenous	uniform composition throughout the sample
Flavour (D+W)	burnt	taste of burnt toast
	sweet	sucrose[5]
	bitter	caffeine[5]
	sour	citric acid[5]
	cereal taste	taste characteristic of cereal (related to rye)[3]
	hay	resembling the taste of dry grass or hay
	salt	saltiness
	roasted	toasted almonds
	off-flavour	unpleasant residual flavour in the mouth[5]
	aftertaste	flavour staying (>1,5 min) in mouth after swallowing[5]
Texture (W)	milk absorption/" soggy"	how much milk the breakfast cereals absorb[3]
Flavour (W)	milk	overall intensity milk taste

*D=dry characteristics, W=bowl-life characteristics

1.(Faller', Faller, & Klein, 2000) 2. (Heiniö, Katina, Alam, Sozer, & Kock,2015.) 3.(Kälviäinen, Salovaara, & Tourila, 2002) 4.(Lenfant, Loret, Pineau, Hartmann, & Martin, 2009) 5.(Karlsen, Aaby, Sivertsen, Baardseth, & Ellekjær, 1999)

2.7.2 RATA evaluation

The sensory evaluation was performed at RISE in individual booths under white light and at room temperature. Each sample was labelled with a three-digit code and presented monadically to the panellists in completely randomized order out of plastic containers along with 20 ml of milk (3% fat per 100g) with corresponding codes for the samples it was intended for. The containers with samples and milk were prepared 1 hr before evaluation. Sample size was 11 pieces which corresponds to 8-11g. Water and cucumber were provided for cleansing the palate in between samples (Figure 8).

Each sample was evaluated first as dry (dry sample characteristics) and then after 3 min immersion in milk (wet characteristics). Clear instructions were given regarding how the evaluation should be carried out including the amount of sample per bite, how the agitation with milk should be done etc. to standardize the procedure. The evaluation was conducted in two replicates with a 10 min break in between. For both odour, texture and flavour panellists were also encouraged to add any additional comment or attribute if perceived in their evaluation form.

Figure 8. Both for panellist including samples, milk, water, cucumber and attribute list.

2.7.3 Statistical analysis

All attributes that were perceived by the panellist were rated on a scale between 1 and 5 which corresponds to the lowest and highest intensity, respectively. The data were collected using a computerised data system (EyeQuestion®, Netherlands).

Overall means for each sample (n=6) from the two replicates were calculated for every attribute using the score data collected. Attributes that were not chosen and scored counted as zero in the analysis. Those obtained means from the categorical data were taken as continuous variables.

A design matrix, X, was created with the attributes in columns and samples in rows with each cell corresponding to the mean score given by the panellists.

The results were analysed by a mixed model two-way ANOVA to obtain influential attribute-product effects. The model included the two main effects: samples and panellists and the interactions between them. Products and panellist are qualitative variables while attributes are quantitative variables. Applying to this model product differences were fixed-effects while panellist differences and panellist-by-product interactions were random-effects.

In case of no product effect for a given attribute, it was removed from further analysis.

Radar charts of dry and wet characteristics for each sample was used to visualize the sensory profile of each breakfast cereal.

Principal component analysis (PCA) was used to visualize the variance among the sensory data to obtain information about the relations between the variables. All statistical analysis was carried out using XLstat (Addinsoft, France) and Rstudio version 1.2.1335 (USA).

3. RESULTS AND DISCUSSION

3.1 Microstructural analysis CLSM

The pore structure, surface structure and arabinoxylan in cell walls were analysed with CLSM. Prototypes were compared to each other based on the following questions: *How BSG affect the microstructure, how fermentation and the possible presence of dextran influence the microstructure* and *how bioprocessing with both LAB and enzymes change the microstructure of the breakfast cereals?* The commercial breakfast cereals, Råg Fras® (Quaker) and All-Bran® (Kellogg's) were analysed to highlight differences encountered among the samples and comparisons between the non-bioprocessed controls were also made to point out disparities between the varieties of BSG.

3.1.1 cross sectional analysis

The cross-sections stained with Texas Red visualizes the pore characteristics of each breakfast cereal prototype.

The micrographs in Figure 9 show the cross sections of each breakfast cereal prototype stained with Texas Red. The sizes, shapes and overall pore structure of the sample are visualized. Magnification 5x and scale bar=500µm.

The sample containing the spent grain (D.BSG) displays a denser and more compact overall structure compared to the reference sample (Rye ref). The reference displays a porous structure with many elliptical pores evenly distributed in the matrix as well as smaller holes and cavities present while the pores of D.BSG have a more irregular and curved shape. The structure of the matrix of D.BSG seems denser. All samples with BSG were less porous than the reference which is explained by the higher fibre content corresponding to incorporation of BSG. An increased fibre content results in a reduced starch concentration which may lead to restrictions in starch gelatinization due to fibre-starch and fibre-water interactions. Also, the fibre particles can cause pre-mature rupture of cell walls before the gas bubbles have expanded to their full potential, which reduces the overall expansion and results in less porous structures with higher density (Oliveira, Alencar, & Steel, 2018). These findings were correlated with literature findings stating that addition of BSG lower the expansion and promotes a structure with small cells in extruded ready-to-eat snacks (Stojceska, Ainsworth, Plunkett, & Ibanoğlu, 2008).

In S.BSG the pores are larger, in various shapes; triangular and elongated compared to S.B+ where the pores appear to be more numerous but smaller. The pore walls of S.BSG seem perforated with many small rounded holes that are not seen for the fermented prototype, at least not as distinct.

The matrix of the S. B+ appear more finely fragmentized in its structure and more merged together. The increased degree of fragmentation seen for the fermented sample may be linked to the action of LAB during fermentation.

The unfermented prototype (D.BSG) also shows a larger and more evenly distributed pore structure compared to the fermented prototypes (D.B+ and D.B-) which appear to have smaller pores. The contours of the pores in D.BSG look more rounded as opposed to D.B- whose pores look "carved out" with more defined contours and sharper edges.

The fermented samples with and without dextran (D.B+ and D.B-) resembled each other's pore structures more than what D.BSG and D.B- did. A possible explanation is that a certain amount of dextran was produced

also in D.B- during fermentation. Fermentable sugars like maltose, glucose and maltotriose may be present in BSG after enzyme digestion during the malting and mashing step in the beer brewing process and serve as nutrients for the LAB. The decrease in pH (Table 4) indicates formation of lactic acid and hence growth of the LAB even though no signs of dextran was seen in those samples. A subtle visual difference seen between the dextran-positive D.B+ and the dextran-negative D.B- is the overall structure of D.B+ matrix appear more fused together which can be a sign of dextran's effect as a hydrocolloid. For all spent grain samples there were areas emitting stronger autofluorescence seen as more highly illuminated regions in the images implying fibre structures present.

Both fermented samples seem slightly more compact compared to D.BSG with smaller pores.

Similar pattern can be seen for the prototypes containing BSG from Peroni. The non-bioprocessed breakfast cereal has more evenly distributed large, irregularly shaped, pores whereas the bioprocessed prototype contains more numerous but smaller pores which gives a denser overall pore structure of the cross-section examined.

However, the bioprocessed sample with BSG from Peroni appear to be more porous compared to the analogues from Dugges and Senson in terms of size and number of pores. The less compact structure can be attributed to the more extensive bioprocessing carried out on this sample including both fermentation and enzymatic treatment which may cause a more successful degradation of larger fibre structures and thus obtaining an airier breakfast cereal.

A more compact structure of the matrix with less pores present is seen for D.BSG compared to the other two BSG varieties; S.BSG and P.BSG. P.BSG appear most porous based on its dispersed and evenly distributed pore network. A possible explanation to the differences observed is the variation in particle size between the BSG varieties. The smaller particle size due to a more efficient milling technique enhance a more porous structure according to findings in the litterature (Sakhare, Inamdar, Soumya, Indrani, & Rao, 2014).

Furthermore, a too wide particle size distribution, like in the Dugges prototypes, can lead to an uneven water distribution and thus counteract expansion (Robin & Palzer, 2015).

Comparing the cross section of the breakfast cereal prototypes with that of a commercial breakfast cereal, Råg Fras® (Quaker), the cross section of Råg Fras is constituted of two walls with an air cavity in between. The material constituting the walls is also very porous in its contexture, having larger pores as well as small holes thus giving it a more porous and airy overall structure compared to the prototypes in this project.

The more compact and less expanded structures seen for the fermented samples compared to their controls can hypothetically be attributed to the presence of dextran. As dextran is a hydrocolloid forming a gel and thus holds water, there is a higher moisture content in the fermented dough compared to the non-fermented. This correlates with finding in the literature that states that higher moisture levels can result in lower expansion ratios and increased hardness of the extrudates (Wójtowicz et al., 2015).

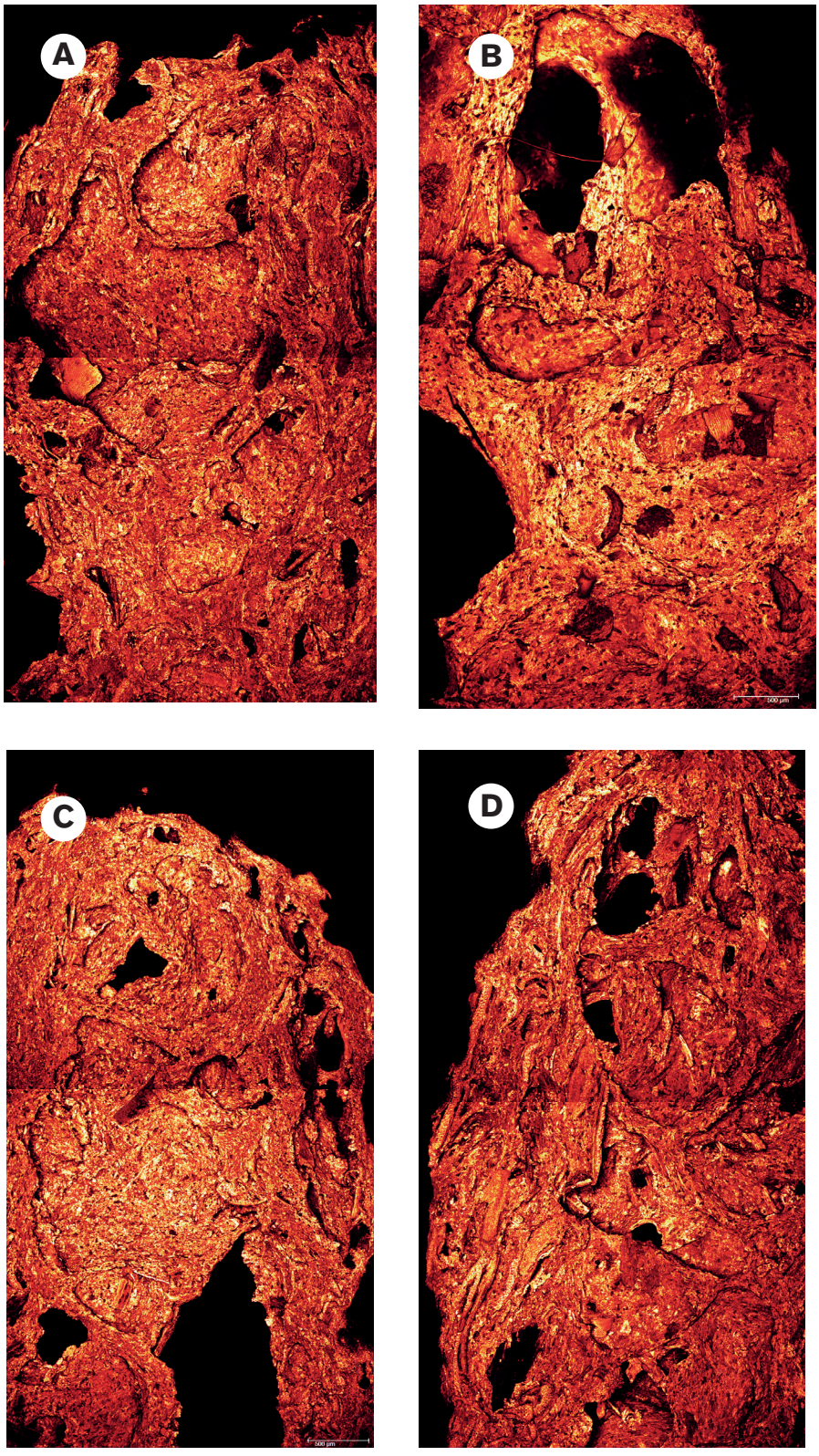
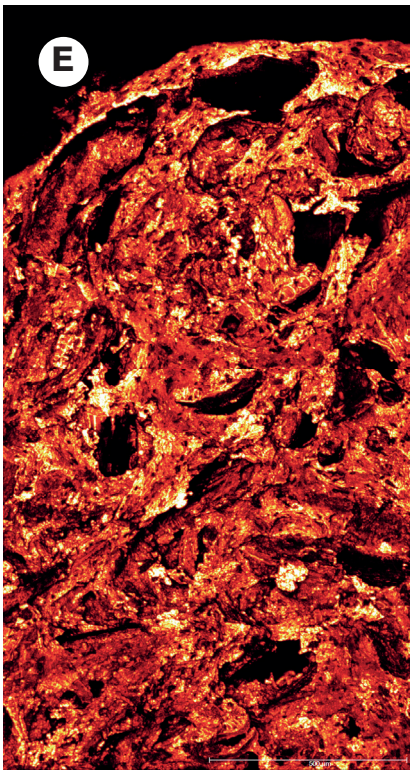
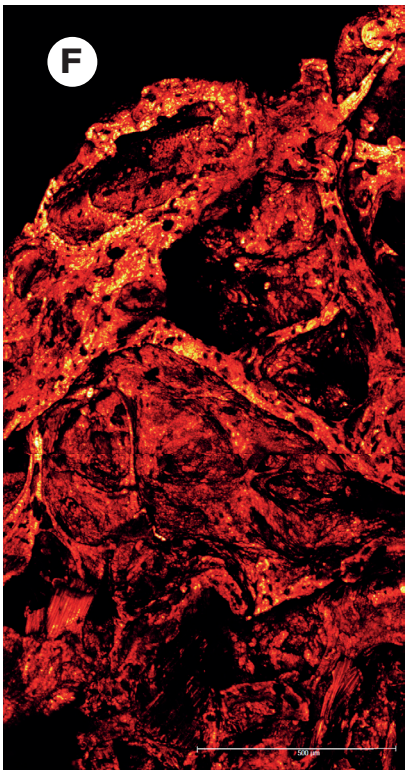
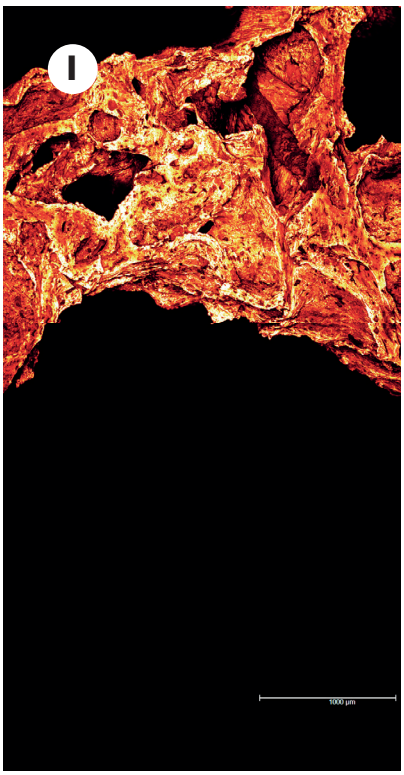
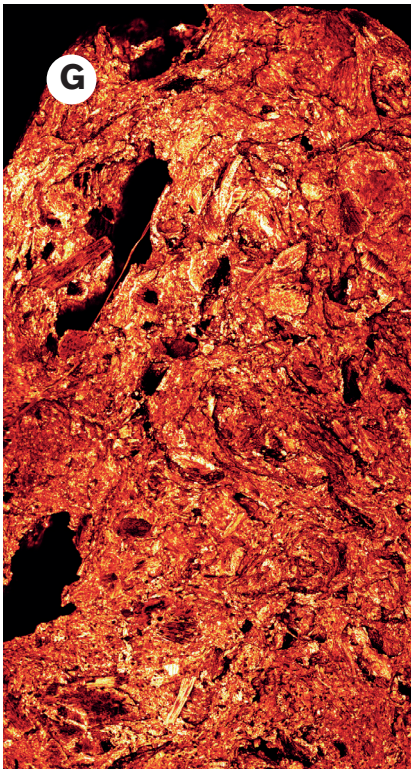


Figure 9. Micrographs of cross sections stained with Texas red and visualized with CLSM. A) D.BSG, B) Rye ref, C) D.B+, D) D.B-
Magnification 5x and scale bar=500 µm









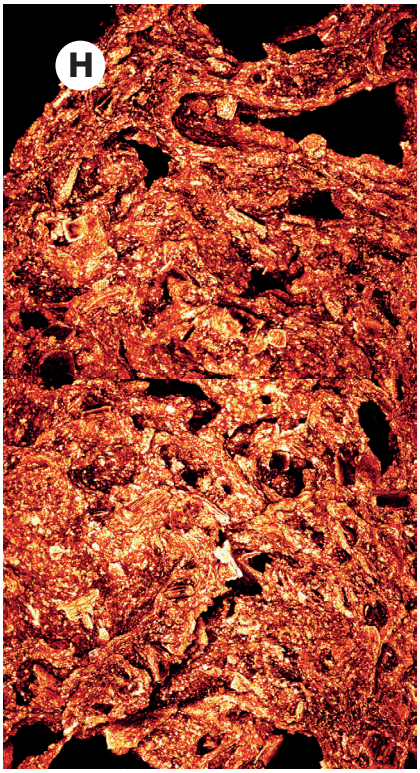


Figure 9. Micrographs of cross sections stained with Texas Red and visualized with CLSM.
E) S.B+, F) S.BSG, G) P.Bio, H) P.BSG and I) Råg Fras
Magnification 5x and scale bar=500 µm.

3.1.2 Surface structure analysis (CLSM)

The surface structure of each prototype was analysed with CLSM for unstained, whole samples. Figure 10 displays the surface structures for all prototypes examined along with the surface structures of Råg Fras and All-Bran. The surface structure is visible and consists of many interconnected fibres in a continuous matrix.

The surface structure of the breakfast cereals containing BSG show clear differences compared to the reference. Visible fibres linked together constituting the continuous matrix for the BSG sample (Figure 10). The surface profile of the reference (Rye ref) contains small adjoined air bubbles seen as black holes in the CLSM image. The matrix of D.BSG thus appear more homogenous and smoother compared to the reference even though larger fibre structures are discernible.

Comparing the prototypes visually with the naked eye the bioprocessed samples; D.B+, S.B+ and P.Bio have a smoother surface structure compared to their controls. A higher degree of decomposition of the particles for those samples may be a reasonable explanation. There are visible fibres interconnected in a longitudinal pattern for all prototypes containing BSG seen as more illuminated structures in the matrix.

The most evident difference coming from autofluorescence between fermented and non-fermented is seen in the samples from Senson where the matrix of the dextran-positive prototype (S.B+) appear more homogenous and fused together with less distinct contours around the fibre structures that constitutes the surface layer compared to its control (S.BSG).

Comparing the prototypes from Dugges the same pattern is seen but less evident. The dextran-positive sample's (D.B+) matrix appear more uniform with less distinct particles compared to the dextran-negative (D.B-) and the non-fermented control (D.BSG) whose matrixes assemble the look of patchworks in their structures. Thus, the surface profiles of D.BSG and D.B- resemble each other more in terms of inhomogeneity compared to D.B+. This finding can either be deduced to the presence of dextran in D.B+ having a merging effect on the surface's matrix thus giving it a smoother surface with increased glossiness or that the particles are more decomposed in this sample.

The same tendency regarding the fibre pattern at the top sheet of the breakfast cereal applied also for the prototypes that were bioprocessed with both LAB and enzyme and its corresponding control (P.Bio and P.BSG). The clear longitudinal fibre pattern seen for P.BSG was not seen for P.Bio whose surface profile appeared more fragmentized with smaller particles fused together constituting its surface matrix.

The structure of the surface differed between the non-bioprocessed prototypes containing BSG from different breweries. The continuous fibre network at the top sheet of the non-fermented Senson prototype had a more distinct patchwork structure compared to the other two non-fermented samples. The longitudinal pattern was more evident for the Peroni prototype. Differences observed can be attributed to different compositions of BSG, bioprocessing and deviations in particle size between the varieties.

The two commercial breakfast cereals Råg Fras® (Quaker) and All-Bran® (Kellogg's) were compared to the prototypes. The surface structure of All-Bran® was constituted of larger fibres fused together without visible pores just like the prototypes in this study. The contours of its fibres were more discernible and its overall surface structure less homogenous compared to the bioprocessed prototypes in this study. The surface structure of All-Bran® resembled the surface structure of D.BSG and D.B- in terms om homogeneity.

The surface structure of Råg Fras is made up of associated small bubbles in a matrix resembling the image of a lunar landscape with craters. Cracked bubbles, visible as round black hole structures, were seen in other samples as well but not as numerous and evenly shaped as in Råg Fras. Larger but fever holes were observed for the rye reference's surface. No surface gloss was observed for any of the commercial samples examined.

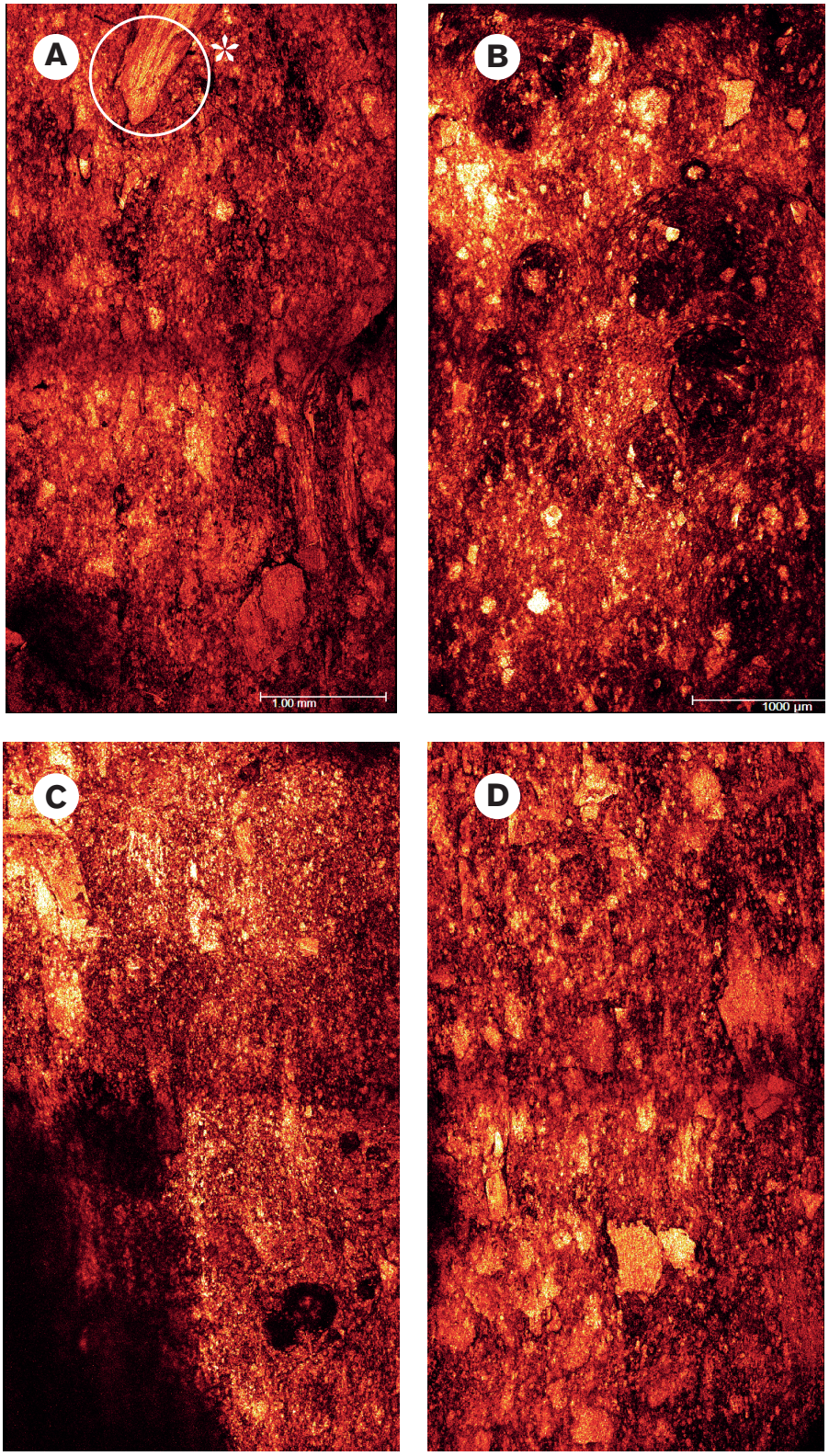


*Figure 10. Surface structures of the prototypes visualized with CLSM. A)D.BSG, B)Rye ref, C)D.B+ and D)D.B- * = example of a fibre structure Magnification 5x and scale bar=1000µm.*

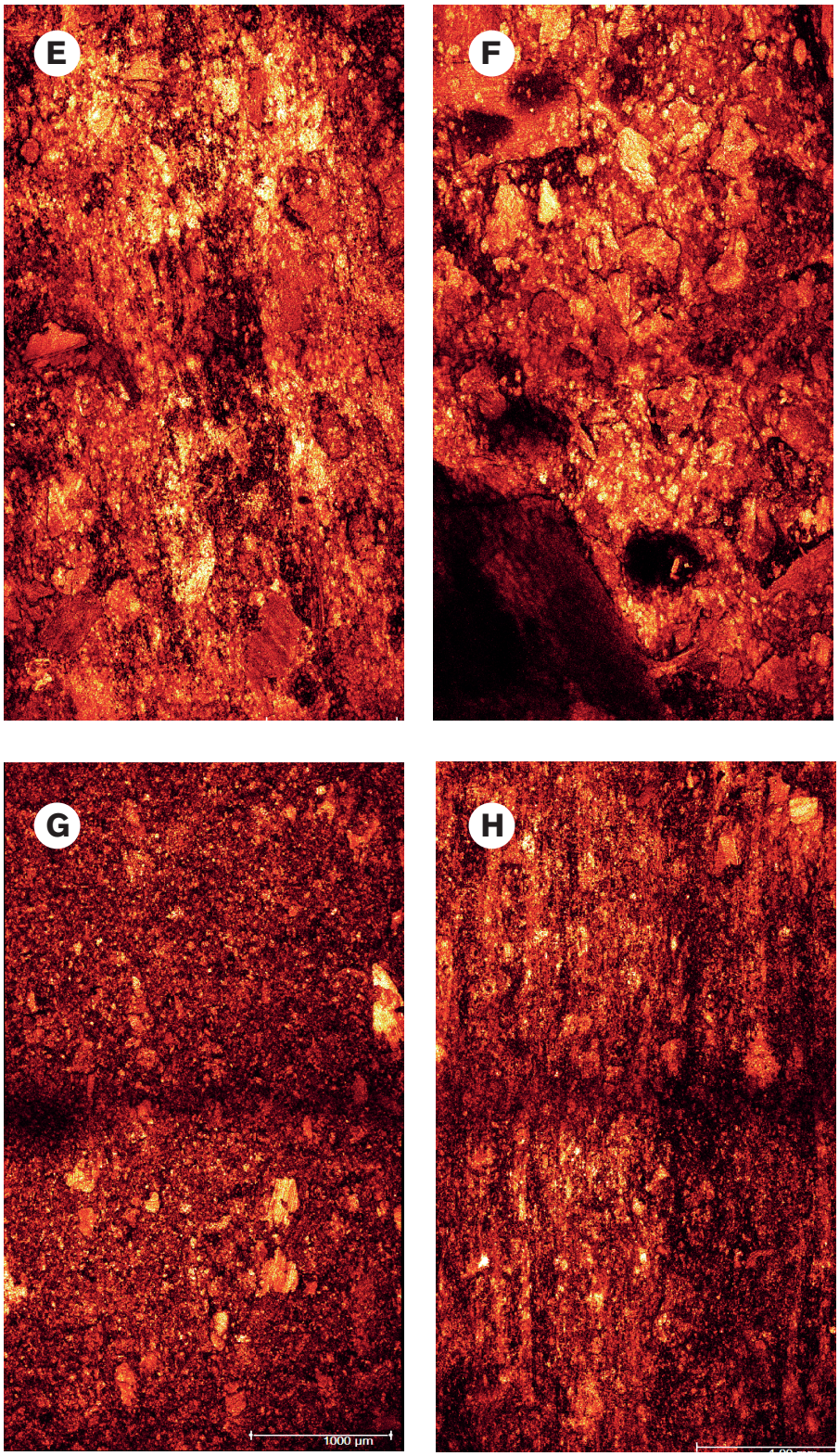


Figure 10. Surface structures of the prototypes visualized with CLSM.
E) S.B+, F)S.BSG, G)P.Bio and H) P.BSG
Magnification 5x and scale bar=1000µm.

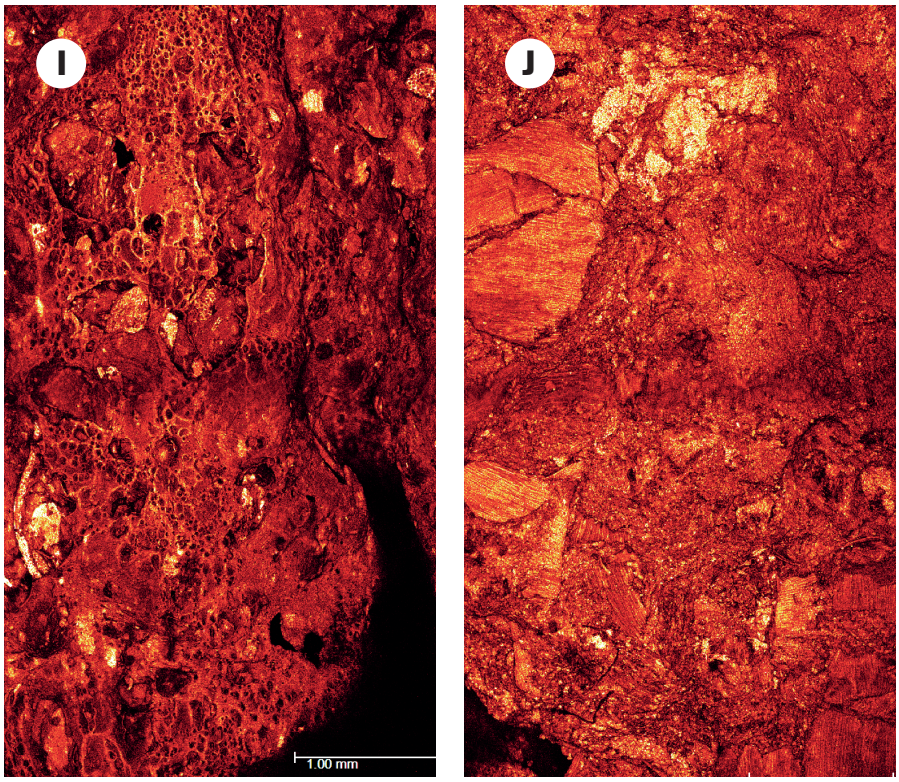


Figure 10. Surface structures of the prototypes visualized with CLSM.
I)Råg Fras. J)All-Bran
Magnification 5x and scale bar=1000 µm

3.1.3 Arabinoxylan (CLSM)

CLSM microscopy of the breakfast cereal sections immunolabelled with LM11 to visualize the effect of bioprocessing on arabinoxylan (AX). AX are shown in red and other material that emit auto fluorescence are shown in green in the micrographs. Multi-layered aleurone structures are assumed to originate from the BSG while single-layered aleurone are assumed to originate from the rye and hence, comparisons were focused on multi-layered structures in the samples. For comparison between aleurone structures in BSG and rye, the reader is referred to section *1.2 Barley*.

The overall matrixes of the fermented samples appear more fragmentized compared to the unfermented sample as they contain smaller and more numerous green auto fluorescent particles in their background matrix. Thus, gives a more scattered overall impression. Larger and less impaired multi-layered aleurone structures were seen for the untreated sample, D.BSG, whereas most multi-layered aleurone structures seen in the fermented samples are smaller when comparing the samples with BSG from the same breweries

No clear difference was observed between the Dextran-positive and the dextran-negative sample with respect to their overall matrixes. Nor differed the thickness of AX in the cell walls. Signs of degradation was seen for both samples, visible as red fragments dispersed throughout the matrix. Degradation from the sub-aleurone region of the endosperm towards the pericarp was observed for aleurone structures in both D.B+ and D.B- with signs of AX spread out from the cell walls of the aleurone to the matrix. A possible explanation to the similarities observed, despite the probable difference of dextran between the samples, is degradation due to endogenous enzymes present in the BSG. Endogenous enzymes like β-glucanases present in the barley are activated and inactivated earlier in the brewing process. Residues of these enzymes present in the BSG may be re-activated in the drying step carried out at 40°C after the fermentation. The decreased pH in D.B- due

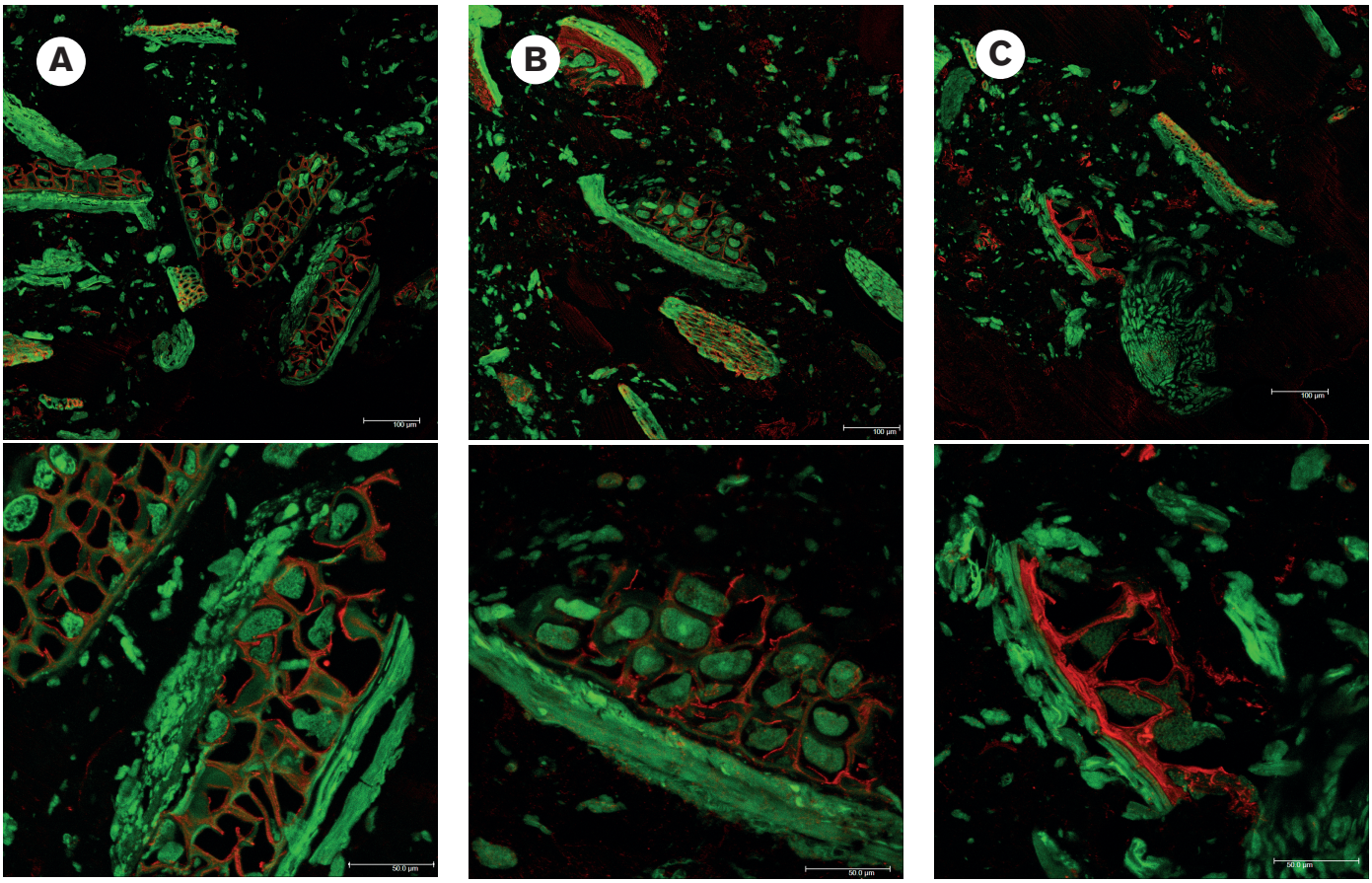


Figure 11. CLSM images of A) D.BSG, B) D.B+ and C) D.B-. Images were collected after immunolabelling of AX. The upper row corresponds to 1x zoom and the lower row to 3x zoom. Magnification 20x and scale bar=100µm and 50µm, respectively.

to fermentation and the optimal temperature range for β-glucanases seem to fairly correlate with values from the literature (Pozen, 1934). Figure 11 displays immunolabelled cross sections of D.BSG, D.B+ and D.B-.

Also, the overall matrix of the dextran-positive sample (S. B+) has a more fragmentized outlook with numerous green particles evenly distributed (Figure 12).

Further, comparing the immunostained cell walls of the fermented sample with its control (Senson) the red signal corresponding to the presence of AX appear less intense for the dextran-positive sample. Also, the thickness of its cell walls seems slightly thinner (Figure 13). This finding can hypothetically be related to degradation occurred during the fermentation process for the LAB to produce dextran.

The AX in the Peroni prototypes correlated with what was seen for the samples from Dugges and Senson but differences in fragmentation for the overall matrix

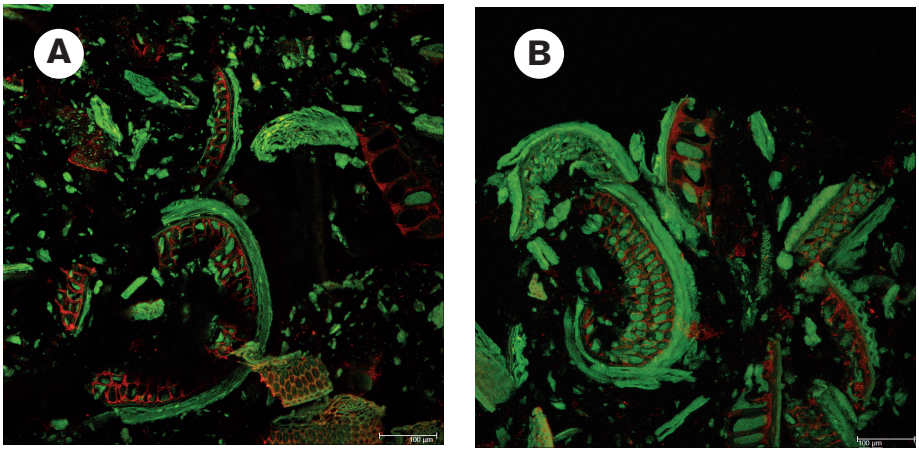


Figure 12. CLSM images of A) S.B+ and B)S.BSG. Images collected after immunolabelling of AX. Magnification 20x and scale bar=100µm.

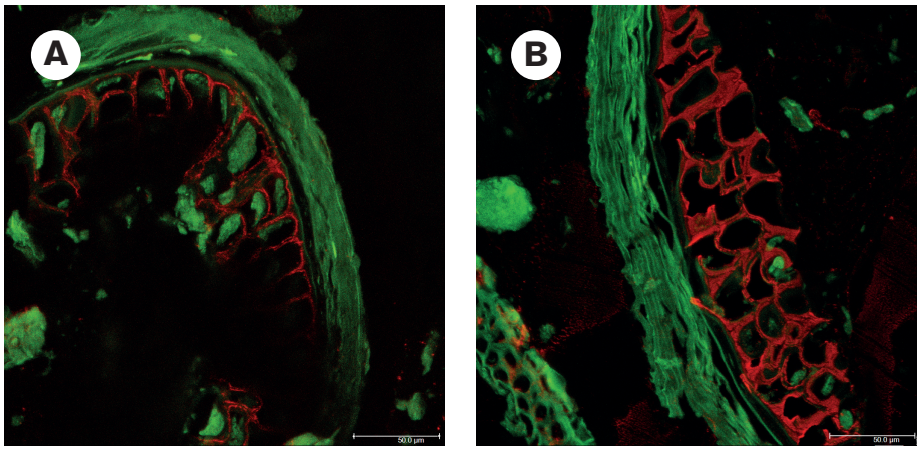


Figure 13. CLSM images of A)S.B+ and B) S.BSG. Images were collected after immunolabelling of AX. Magnification 20x, zoom=3x and scale bar=50µm.

between bioprocessed and non-bioprocessed was not as evident for those samples (Figure 14)..

On the other hand, a more prominent degradation of AX was observed for the bioprocessed sample (P.Bio) compared to the non-bioprocessed sample (P.BSG). P.BSG appear to have thicker AX-containing cell walls, more intensively stained (red), with more intact cell walls compared to the cell walls of P.Bio where the cell walls seem more decomposed and AX are seen dispersed in the matrix, visible as red stripes in the black matrix.

The overall trend observed for most samples with more prominent signs of degradation for the bioprocessed samples may indicate an increased bioavailability of the phenolic substances bound to AX through the fermentation process as the AX is seen to be released from the cell walls.

This difference between fermented and control in terms of fragmentation was most evident in the Senson prototypes. The smaller particle size and hence more effective fermentation process has most likely caused a more efficient fragmentation. It is also reported in the literature that smaller particle size facilitate the release of compounds bound to the matrix of the bran (Heiniö et al., 2015.).

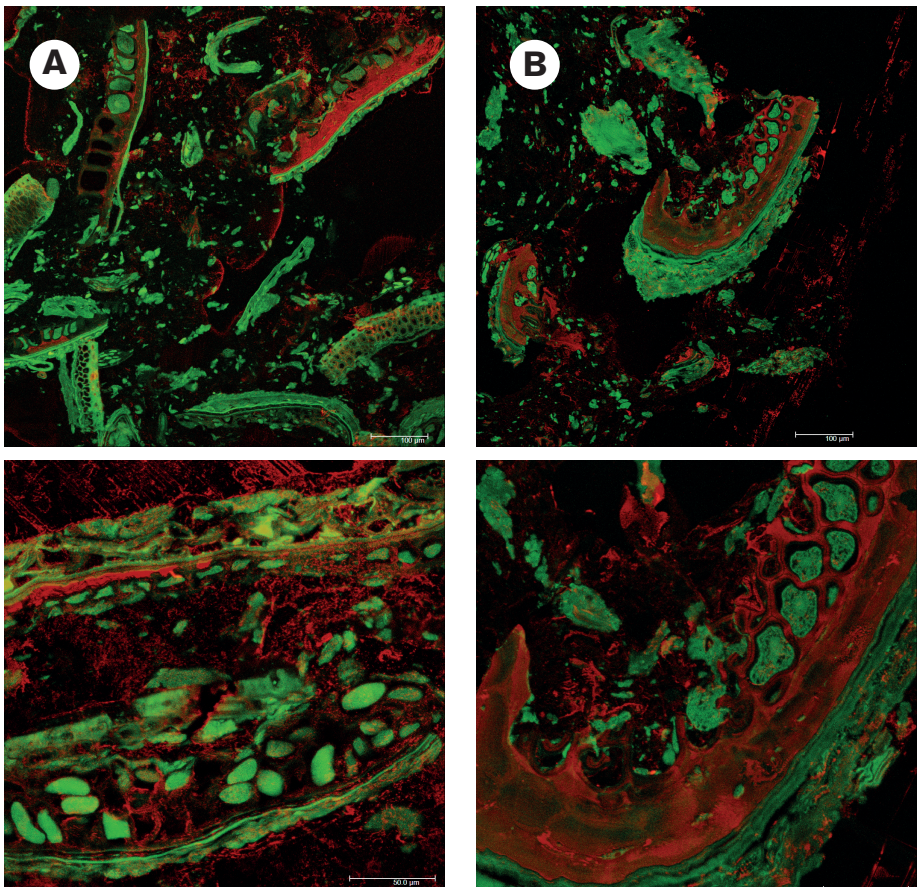


Figure 14. CLSM images of A) P.Bio, B) P.BSG. Images were collected after immunolabelling of AX. The upper row corresponds to 1x zoom and the lower row to 3x zoom. Magnification 20x and scale bar=100µm and 50µm, respectively.

3.2 Microstructure analysis Light microscopy

Protein and starch were visualized using LightGreen and Lugol's iodine (1:10) in brightfield mode. Light Green stains protein green whereas the amylose in starch is stained blue, amylopectin brown/pink and protein yellowish by Lugol's (Nikinmaa, Mattila, Holopainen-Mantila, Heiniö, & Nordlund, 2019). A mixture of those stains was used to obtain a comprehensive image of the matrix. Cross sections analysed were either cryosections obtained pre-microwave-oven (10 µm) or microtome sections obtained post-microwave puffing (1 µm).

Overall morphology

For all samples examined amorphous starch constitutes the continuous phase with proteins and bran particles distributed within.

Comparing the light micrographs for the sample containing BSG from Dugges brewery (D.BSG) with the reference (Rye ref) there are larger and more numerous fibre structures present in D.BSG even though there were some fibres seen also in the reference. Also, the structure of the starchy matrix differs with respect to the starch granules. The granules seen in D.BSG seem smeared out and merged thus giving it a more homogenous outlook whereas the granules in the reference are more shaped like irregular tubules with defined contours (Figure 15).

Furthermore, the protein clusters differ in shape and number between the samples observed and denser protein clusters was seen for D.BSG. The shape of the protein particles seen in the reference sample was more elongated.

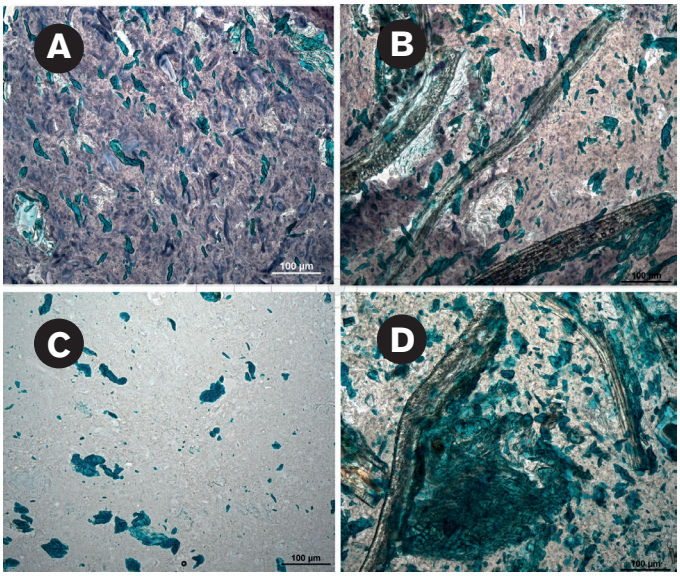


Figure 15. LM micrographs (10x) of. A) Rye ref, B) D.BSG, C)Rye ref and D) D.BSG. Upper row stained with LightGreen and Lugol's mixture, lower row stained with only LightGreen. Bar=100 µm

Pores

White unstained areas in the samples are pores which are seen for all samples and constitutes the discontinuous phase. The pores differ in size and shape. The overall pore structure is more compact for the BSG-samples with smaller pores less evenly distributed compared to the reference where larger and more numerous pores are seen. Furthermore, the shape of the pores differs as the larger fibres and bran particles in the BSG-prototypes seem to break up the starchy matrix and cause pores due to cracks. S.BSG has a broken matrix structure with pores appearing longitudinally along large fibres whereas more rounded pores are seen in the reference (Figure 16). The higher fibre content attributed to the BSG can either have a reinforcing effect or promote brittleness in the product depending on how the fibres interact with the continuous phase (Chanvrier et al., 2013). According to findings in the textural- and sensory evaluation of these products the addition of BSG seemed to have a reinforced effect making these products more compact and harder. A more compact structure was observed for the BSG-prototypes also in the cross-sectional examination in the CLSM (section 3.1.1).

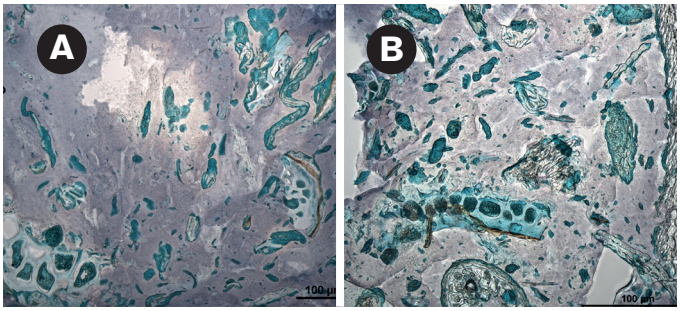


Figure 16. LM micrographs visualizing pores and fibres in A) Rye ref, B) S.BSG. 10x magnification.

Another difference observed in both CLSM and LM between the differently bioprocessed samples was the pores' shape. Sharper contours and more edgy pores were seen in D.B- compared to D.B+ that had more rounded pores (Figure 17).

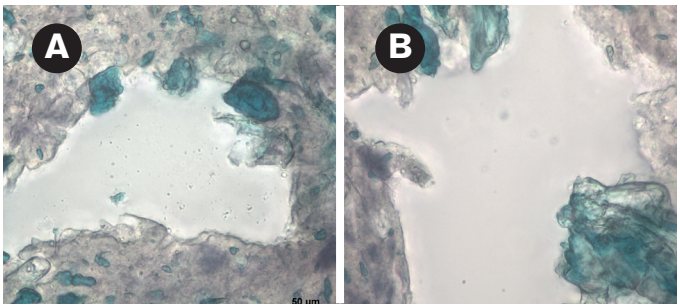


Figure 17. LM micrographs visualizing pore shape (40x) in A)D.B+ and B) D.B-. Bar= 100 µm.

Protein distribution

The protein distribution, visualized by staining with LightGreen, was more aggregated in cohesive cluster structures in the un-fermented controls compared to the fermented samples. The protein network is fragmentized for all samples examined, due to mechanical damage during milling and extrusion cooking, but the protein network appears more fragmentized and finely dispersed throughout the matrix in the fermented samples. The overall increased fragmentation seen for the bioprocessed samples was seen also in the cross-sectional analysis (3.1.1) and AX-analysis (3.1.3). These findings indicate that degradation of cell wall structures around the proteins have taken place. Protein bodies that had been enclosed by the cell walls seem to have been released and dispersed in the starchy matrix. This may be attributed to the lowered pH caused by the LAB during fermentation which activates proteolytic enzymes that can cause depolymerization of proteins (Wang et al., 2018). Untreated samples with BSG from Senson was shown to have a smaller particle size distribution compared to Dugges and hence, a more effective fermentation and a more fragmentized protein network which further strengthens this hypothesis. An increased dispersion of protein particles was seen also in the dextran-negative sample (D.B-), which is in line with the hypothesis too as proteolytic enzymes may have been activated in this sample due to the lowered pH after fermentation. Figure 18 visualizes the protein distribution for the Senson prototypes.

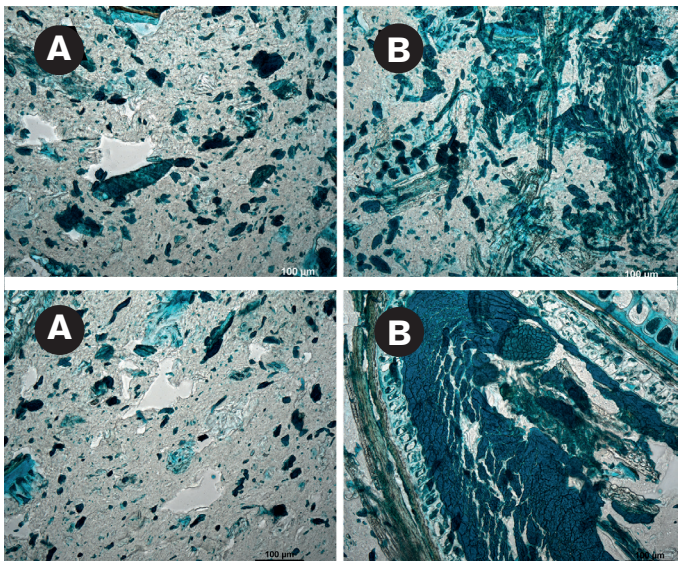


Figure 18. LM micrographs displaying the protein distribution for the Senson prototypes. Upper row=10x and lower row=20x. A) S.B+ and B) S.BSG. Scale bar=100 µm.

Fibres and cell wall degradation

Comparing the overall matrix structure for the fermented samples along with their respective controls revealed some differences with respect to their fibre content. Smaller fibre fragments were observed in the fermented Senson prototype compared to its non-fermented control (Figure 19).

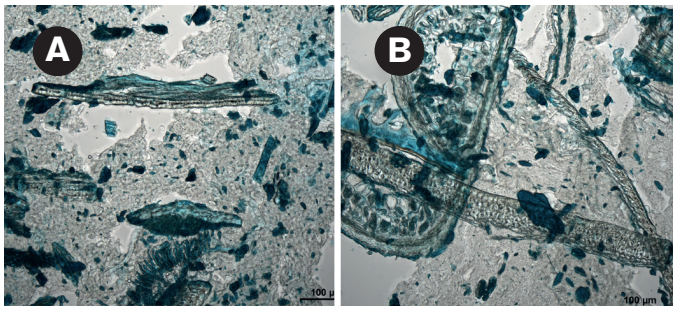


Figure 19. LM micrographs 10x displaying fibre structures in A) S.B+ and B) S.BSG. Scale bar=100 µm.

Comparing the size of the fibres between the dextran-positive and the dextran-negative samples from Dugges displayed smaller fibre structures in the dextran-positive sample (D.B+) comparatively to its dextran-negative control (D.B+). Fibres in the Dugges prototypes are visualized in Figure 20. The differences observed are assumed to be attributed to the influence of LAB and dextran during fermentation.

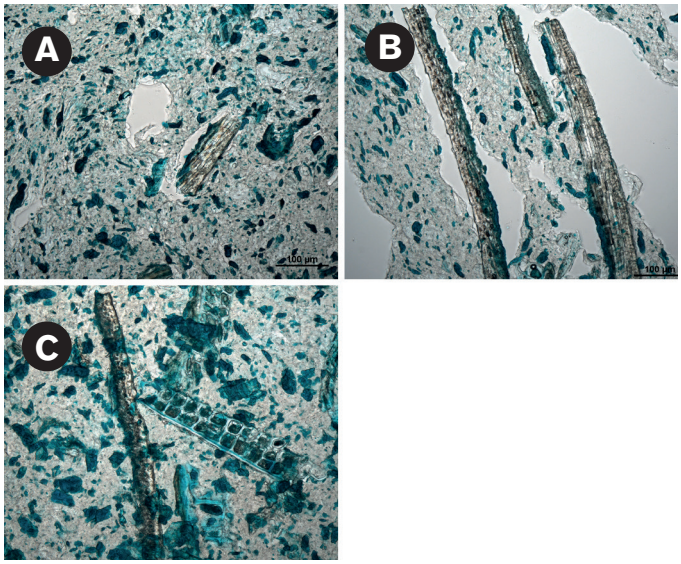


Figure 20. LM micrographs 10x displaying fibre structures in A) D.B+, B) D.B- and C) D.BSG. Scale bar=100 µm.

Differences encountered between the non-bioprocessed controls containing different kinds of BSG could be attributed to variations in BSG composition and deviations in particle size and hence, varying occurrence of larger fibre fragments disrupting the continuous starch matrix. Larger fibre structures were seen for the prototypes from Dugges and Senson compared to the Peroni samples which correlates well with its particle size distribution as well as, sensorial and textural differences revealed (Figure 21).

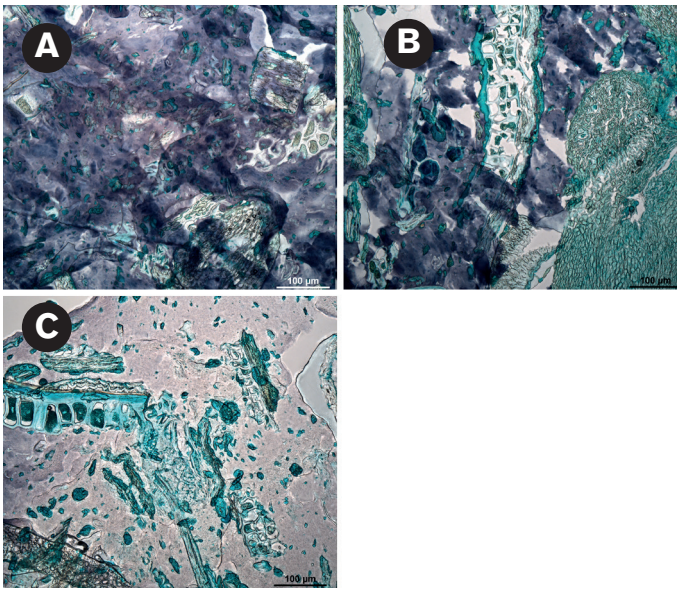


Figure 21. LM micrographs (10x) of non-bioprocessed control prototypes with BSG from different breweries. A) P.BSG, B) D.BSG and C) S.BSG. Scale bar=100 µm.

There were signs of cell wall degradation observed to some extent for all BSG samples examined. In both dextran-positive prototypes (D.B+) and dextran-negative prototypes (P.Bio) protein bodies were seen that had leaked out from the aleurone cells to the matrix and some cell walls were dissolved. This occurrence seemed however more common in the bioprocessed samples than their controls. Figure 22 displays cell wall degradation observed in prototypes containing BSG from Dugges and Peroni.

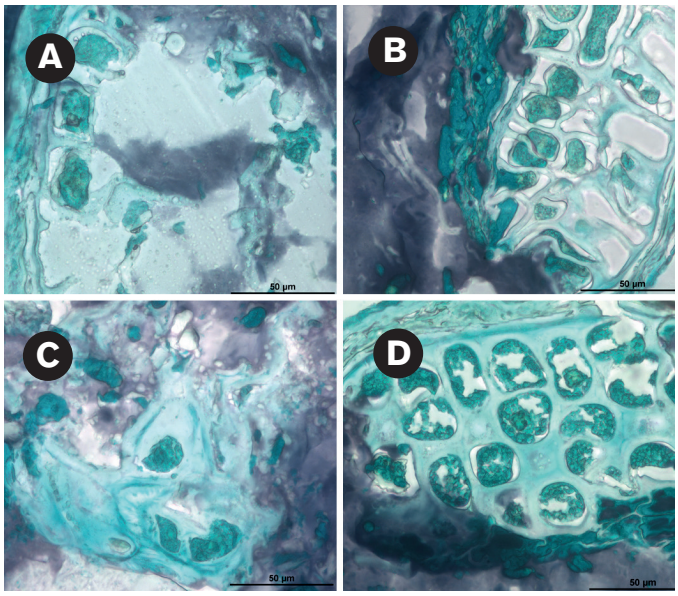


Figure 22. LM micrographs 40x visualizing partly dissolved aleurone structures for A) D.B+, B) D.BSG, C) P.Bio and D) P.BSG. Scale bar=50µm.

Starch

The starchy matrix appears more homogenous and fused together for S.BSG whereas in S.B+ amylose is seen as blue irregular tubular structures (Figure 23). This difference can possibly be attributed to the gel network formed by the polysaccharide that may have a cohesive effect on the starch granules during the extrusion cooking and affect the gelatinization degree of the starch.

Amylose encapsulated within swollen starch granules were seen for both samples but leakage of amylose from the granules were more commonly seen for the fermented sample with BSG from Senson. Amylose leakage can lead to increment of the surrounding solution's viscosity as more water is absorbed by the starch granule when amylose leaks out (Eriksson & Eriksson, 2012).

A hypothesis is that the amylose leakage and hence, increased water absorption had a negative impact on textural attributes like hardness and compactness of the sample based on the results from the textural and sensory analysis. However, further research concerning the dextran's effect on gelatinization, retrogradation and amylose leakage of starch during extrusion cooking is needed.

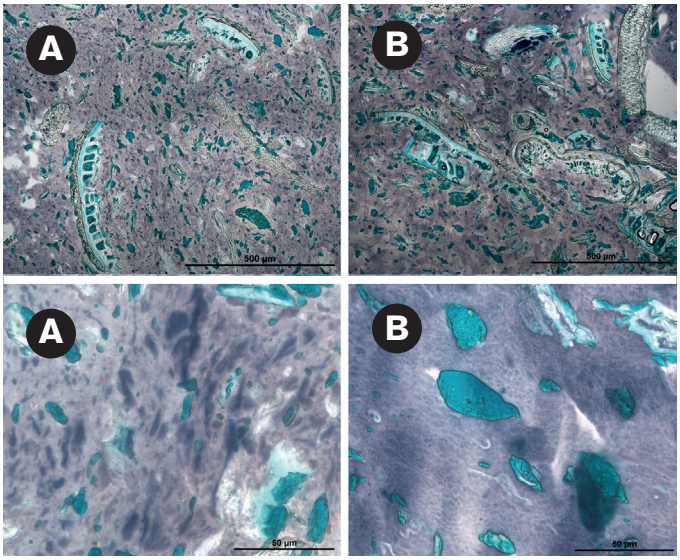


Figure 23. LM micrographs 10x (upper section) and 40x (lower section) visualizing the starchy matrix of the Senson prototypes: A) S.B+ and B) S.BSG. Scale bar=100µm.

3.3 Texture analysis (Instron)

Textural characteristics were measured with an Instron 5542 universal testing machine. Hardness was defined as compressive stress at maximum compressive load [MPa] while the total number of force peaks, Npeaks, along the curve was taken as a measurement for how many layers constituting the breakfast cereal that the probe punctuated and related to crispiness of the product (Valles Pamies, Roudaut, Dacremont, Le Meste, & Mitchell, 2000). The results are expressed as the average of ten measurements.

Young's Modulus (E) seen as the magnitude of the slope of the first peak in compressive strain (%) versus compressive load (N) graphs was used to interpret the stiffness of the breakfast cereals (Figure 24). A crispy or hard product has a high modulus while a soggy product has a low modulus. The first peak corresponds to the force required to break the surface of the sample and hence its surface hardness.

The height of the most puffed part of each sample [mm] was taken as a measure of expansion.

A completely objective assessment could not be made due to the variability within the samples. Further, to distinguish peaks from noise in the graphs and judge

the significance of the peaks is a subjective assessment as no set force threshold was defined for the peaks. However, general patterns observed for the breakfast cereal prototypes were interpreted and analysed in this part of the project.

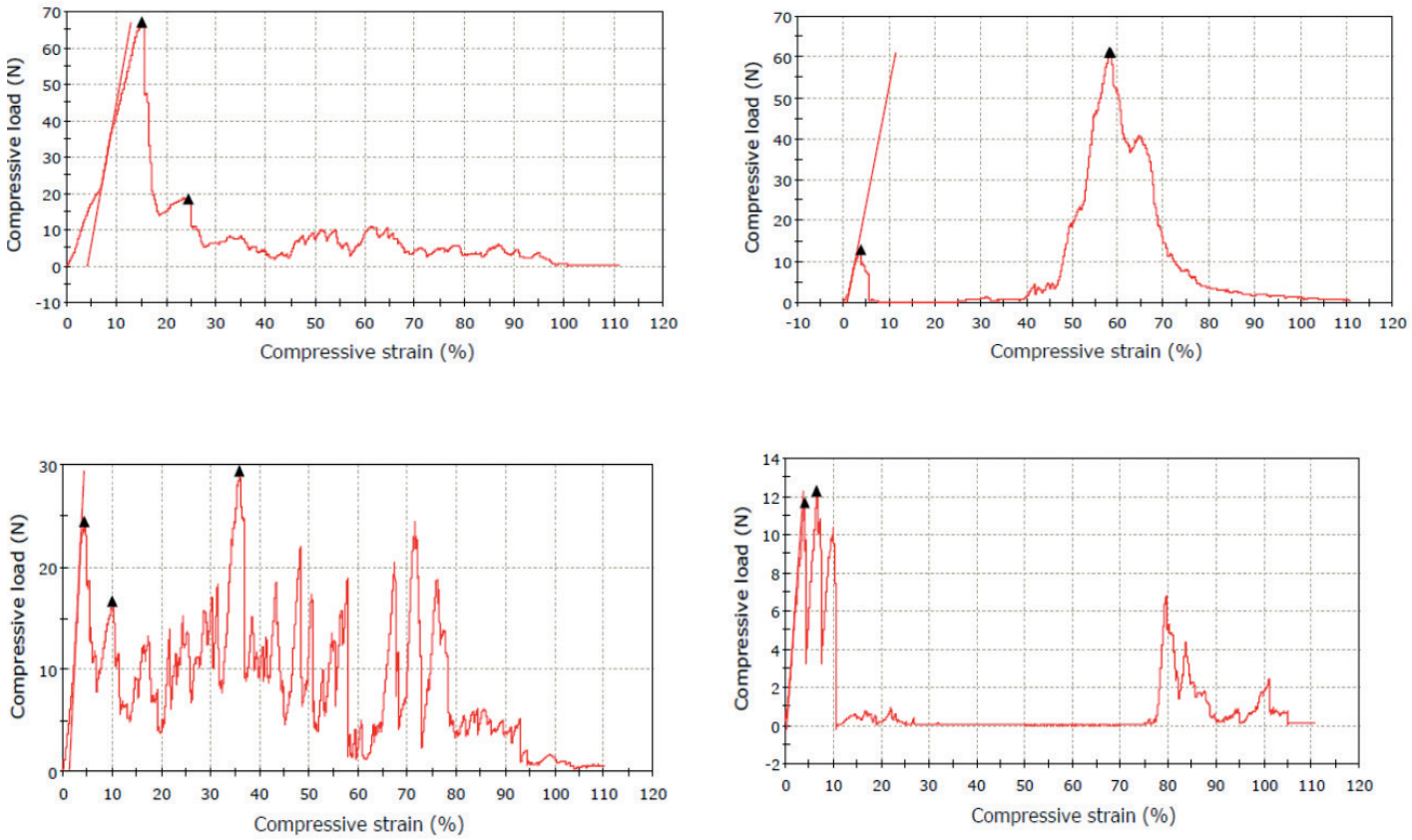


Figure 24. Typical graphs of compressive strain (%) vs. Compressive load (N). Upper row from left: D.BSG and S.B+. Lower row from left: Rye ref and Råg Fras.

3.3.1 Instrumental texture dry samples

Hardness

The hardness ranged from 12,8 to 7,9 MPa for the investigated prototypes with an average standard deviation of 2,0 which indicates a relatively large variation within sample type. Figure 25 visualizes the overall trend in hardness for the different prototypes as well as a comparison to the two conventional breakfast cereals. No significant difference ($p<0,05$) was observed for the bioprocessing treatment, i.e. (D.B+, D.B-, S.B+ and P.Bio) when compared to the non-bioprocessed (D.BSG, S.BSG and P.BSG) with an independent two-sample student's t-test assuming unequal variances.

Compressive stress at maximum compressive load [MPa]

Figure 25. General trends observed in hardness [MPa] of the breakfast cereal prototypes.

A One-Way ANOVA was done to test whether the BSG obtained from the different breweries differed (Table 7). With 'breweries' as a factor the hypothesis tested was:

H0: All individual batch means are equal

Ha: At least one batch mean is not equal to the others

Table 7. ANOVA to test for differences between BSG.

Source of variation	SS	df	MS	F	p-value	F-crit
Between groups	56,39	2	28,19	7,00	0,0035	3,35
Within groups	108,71	27	4,03			
Total	165,10	29				

Since the F statistic was greater than the critical F-value (F-crit) the null hypothesis was rejected, and it was concluded that there is a significant difference between the BSG from the different breweries ($p<0,05$). Pairwise t-tests (two-sample student's t-test assuming unequal variances) were done that revealed a significant difference ($p<0,05$) between BSG from Dugges and the other two breweries. A probable explanation is the difference in particle size due to different milling techniques applied for the different BSG batches which may have a larger impact on textural properties than the bioprocessing treatment itself. Large particle size implies large fibre fragments present that may interfere with the starch and protein in the sample's matrix and thereby affect its structural and mechanical characteristics.

The total mean for all samples containing non-proces-

sed BSG compared to the reference (Rye ref) revealed a significant difference ($p<0,05$) with student's t-test. Thus, the conclusion that an addition of 30% BSG (w/w) had a significant effect on the hardness measured could be deduced. The increased hardness seen for the BSG prototypes are in line with findings in the literature that reports increased thickness of air cell walls and increased hardness in extruded products with high fibre content (Alam et al., 2014). Råg Fras and All-Bran were significantly less hard compared to the prototypes in this study, most likely due to smaller particle size distribution and less fibre content.

A typical compressive strain (%) versus compressive load (N)-graph of two hard prototypes, D.BSG and S.B+, compared to the graphs of Rye ref and Råg Fras is shown in Figure 24. The patterns visualized in the graphs show the general trends observed for those samples. The most evident differences seen is the numbers and shapes of the peaks. Rye ref, followed by Råg Fras, has the highest number of peaks observed compared to all other samples examined. The peaks of Rye ref and Råg Fras are sharper than other samples observed. As the area under the curve can be related to energy required for a given displacement or puncture of the sample, the peaks' sharpness is interpreted as an indication of crispiness and brittleness (Anton & Luciano, 2007). A brittle product may have a high modulus, thus being very stiff, but when broken the force decreases immediately which amounts to a sharp peak. This implies that the lamellas in between the air bubbles are thin in those samples.

The measured hardness, i.e. compressive stress at maximum compressive load [MPa], appeared to correlate negatively with the expansion (R^2=-0,52). This means that more compact breakfast cereals had a relative higher resistance to penetration by the probe. The least expanded samples were the non-fermented controls for Senson and Dugges (S.BSG and D.BSG) which suggest that the particle size is an influential factor for expansion as those had wider particle size distributions compared to the non-bioprocessed sample from Peroni.

Stiffness

A positive correlation (R^2=0.66) was observed between hardness and stiffness (compressive stress a tmaximum compressice load and modulus) for the prototypes. Averaged values for the modulus ranged between 237,0 MPa for Rye ref and 123,0 MPa for P.BSG among the prototypes whereas Råg Fras and All-Bran's averaged modulus were 67,0 and 9,4 MPa, respectively (Figure 26).

The modulus can be related to the force required for deformation of a material. No significant differences could be observed between fermented and non-fermented samples, nor between the different BSG through t-test and analysis of variance tests (ANOVA). However, comparing the total mean for all samples containing non-processed BSG to the reference without BSG (Rye ref) a significant difference ($p<0,05$) was proved (student's t-test). Hence, the incorporation of 30% BSG (w/w) affect the stiffness of the final product by lowering the modulus and thus making it more compressible. The two commercial samples had significantly lower modulus compared to the prototypes.

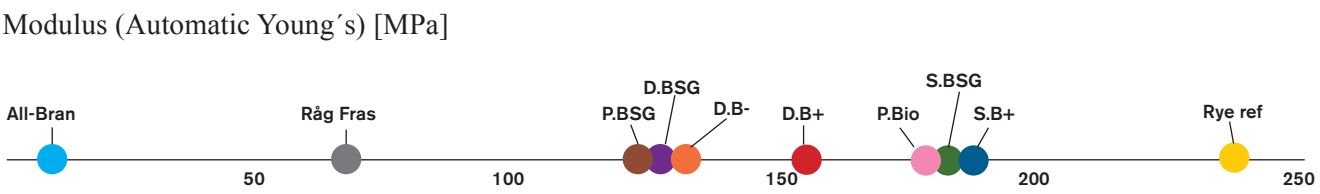


Figure 26. General trends observed in Modulus [MPa] of the breakfast cereal prototypes.

Npeaks

The number of peaks observed for the breakfast cereal samples differed between the samples and their averages ranged from 4.3 to 9.9 corresponding to S.BSG and Rye ref, respectively. The commercial breakfast cereal, Råg Fras, had an average Npeak of 9.5. Since the shape of All-bran differed from the others, this cereal was excluded from this analysis.

A strong positive correlation (R^2=0.91) between Npeaks and the measured expansion was seen (Figure 27). The number of peaks can be related to crispiness as a crispy product consists of many thin layers with possibly more numerous air pockets in between layers. More layers of material constituting the sample thus requires more space and hence a greater expansion. On the other hand, a hard and dense product has thick lamellas in between air bubbles and thus fewer air bubbles.

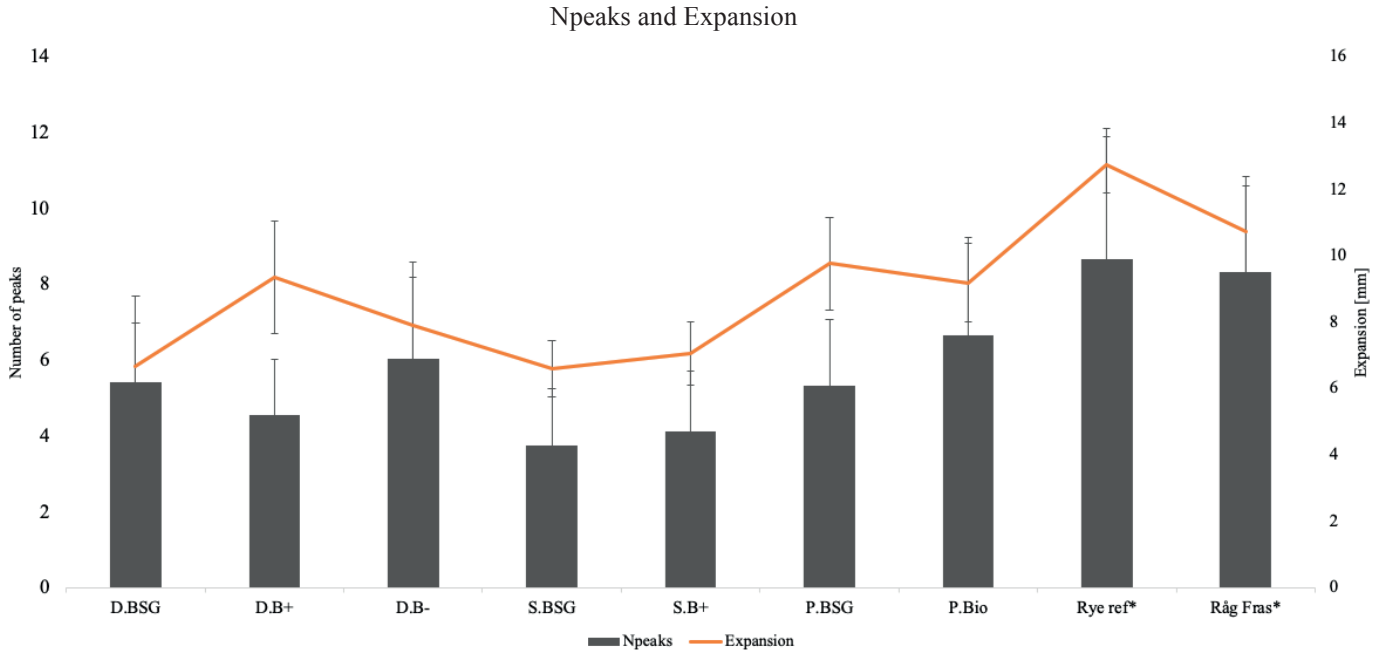


Figure 27. Npeaks related to expansion for the prototypes examined.
**Significantly different (p<0,05) compared to the BSG-containing prototypes.*

3.3.2 Instrumental texture bowl-life

Hardness

Immersion in milk for 10 min led to reduction in maximum compressive stress for all samples except Rye ref. This deviation could be explained by either within-batch variations, less large fibre structures present that disrupt the matrix and may affect the milk's ability to penetrate the sample or that they got tougher and thus required more force to penetrate after soaking.

The percentual reduction in hardness between dry and wet samples for the BSG containing prototypes varied between 9,4% and 56,6% for D.B- and D.B+, respectively. The commercial products, Råg Fras and All-Bran, displayed a reduction in hardness of 76,8% and 96,8%, respectively (Figure 28).

Although pairwise t-test between bioprocessed and non-bioprocessed prototype within the different breweries-categories did not prove any significant difference ($p<0,05$), the pattern visualized indicated that the fermented samples were more affected by the soaking (Figure 28). The bioprocessing with LAB (and enzymes) appeared to facilitate the milk absorption which possibly could be related to findings in the microstructural analysis of the surface structure. A more finely dispersed surface structure was seen for the fermented samples in general.

The reduction in hardness caused by the immersion in milk correlated weak positively with expansion ($R^2=0,24$). Most likely due to that milk penetrates an airier structure easier than a compact structure. Those hypotheses seem valid as the commercial samples were shown to be significantly more affected of the soaking and have both smaller particle size distributions and airier structures compared to the prototypes.

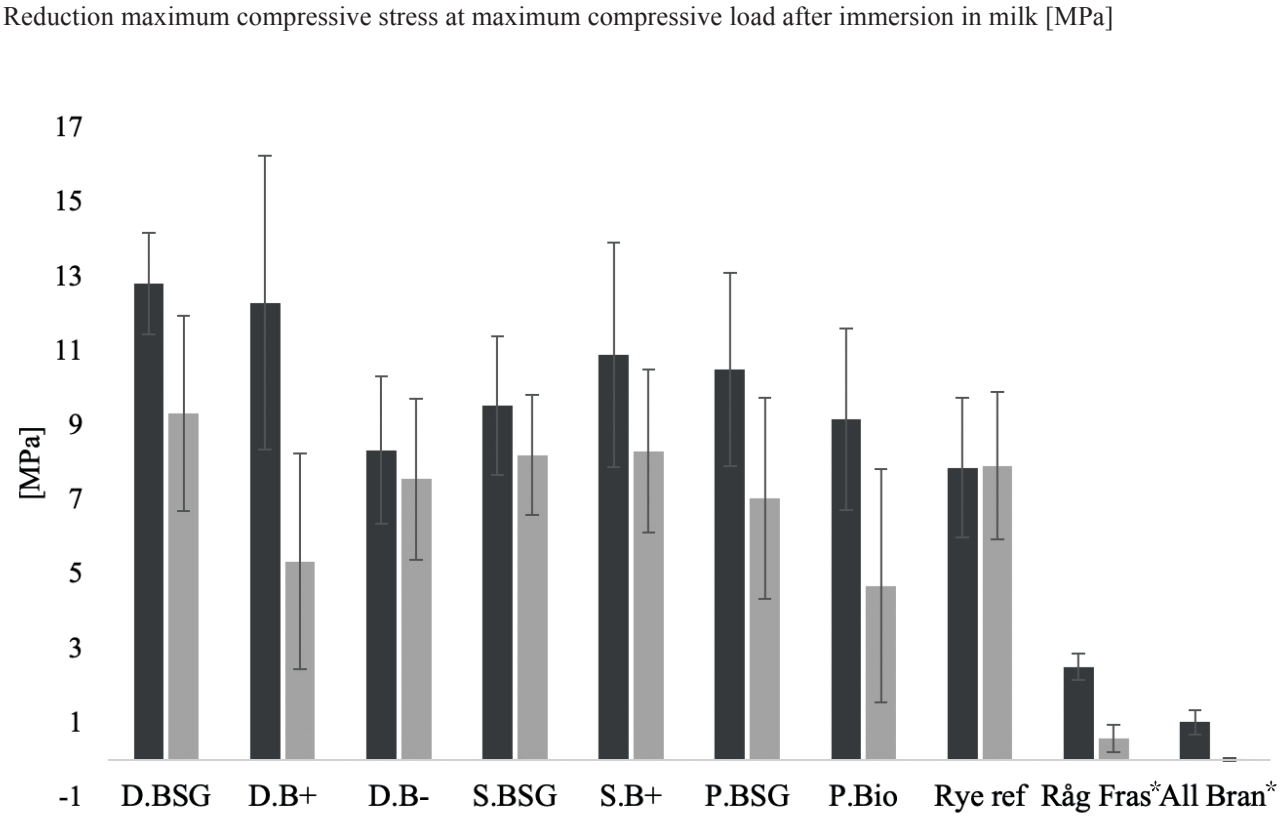


*Figure 28.Reduction in maximum compressive strain at maximum compressive load [MPa] after 10min immersion in milk. Dark grey= dry samples, light grey= after soaking in milk. *=significantly different ($p<0,05$) compared to the BSG containing prototypes.*

Stiffness

A reduction in the modulus after soaking was observed for all samples tested ranging from 42,8% to 80,7% for the prototypes (D.BSG and P.Bio) compared to values of 93,2% and 97,6% for Råg Fras and All-Bran, respectively. Significant reduction in stiffness between dry samples and wet samples ($p<0,05$) was observed for all cereals tested. No significant difference ($p<0,05$) was observed between fermented prototype and their respective control, nor between the different BSG varieties. Figure 29 displays the reduction in modulus for the samples examined.

The immersion in milk was found to decrease the modulus, i.e. less force required to cause deformation to the sample. The breakfast cereals got less crisp. This can be deduced to the milk's plasticization of starch and proteins in the cereals that makes them more flexible and soggier in their texture (Sacchetti, Pittia, & Pinnavaia, 2005).

The prototypes retained their crispiness better after milk soaking compared to the commercial samples tested. Thus, makes them suitable for slow eaters as they are less soggy at the end of the breakfast meal. The trend observed was that the bioprocessed cereals' crispiness was more influenced by the soaking than their respective non-bioprocessed controls, just like for hardness.

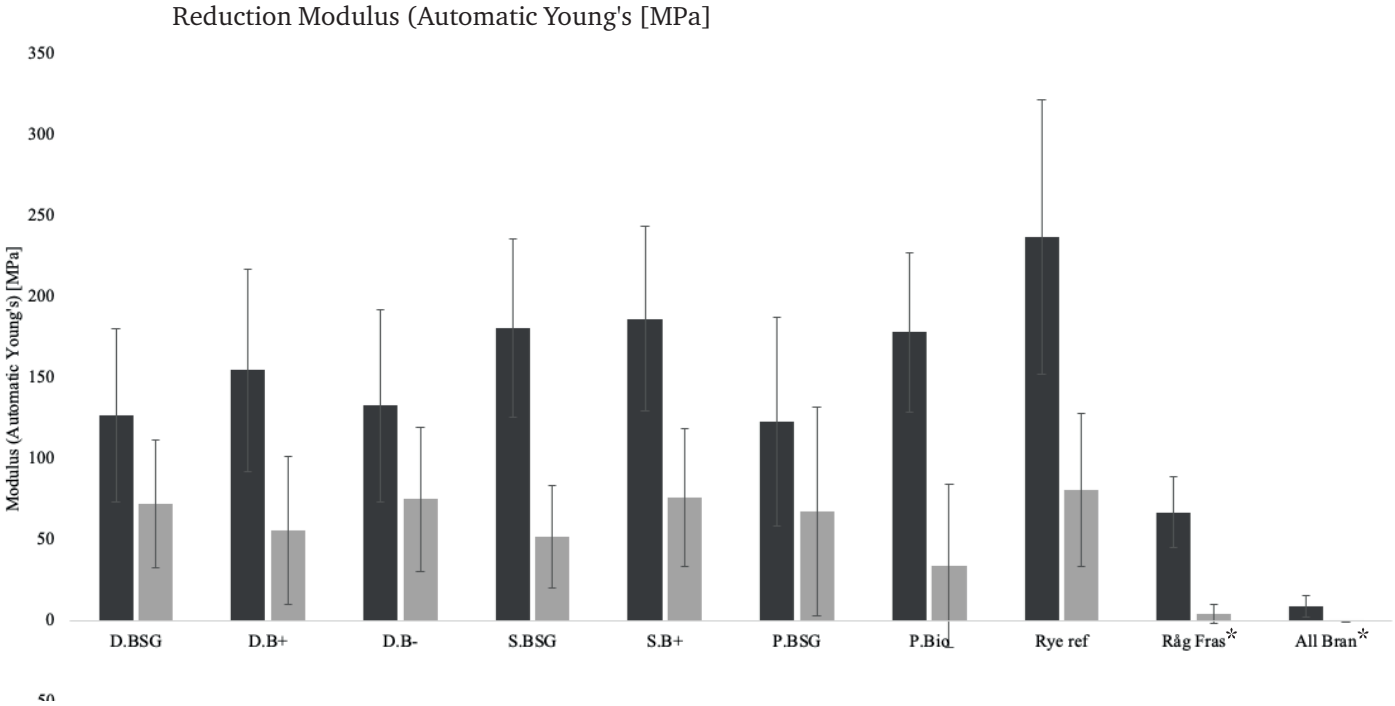


*Figure 29. Reduction in modulus [MPa] after 10min immersion in milk. Dark grey= dry samples, light grey= after soaking in milk. *=significantly different ($p<0,05$) compared to the BSG-containing prototypes.*

3.4 Particle size evaluation

The results of the particle size distribution based on flour fractionation analysis are displayed in Table 8 and Figure 30. Ranking the sieves from five to one with the smallest ranked as number one. In sieves with smaller pore size, larger fractions of finer particles are collected. Fractions larger than 500µm, i.e. residues from sieve number 5, 4 and 3, are considered coarse fractions in this study. The samples retained on the bottom plate, thus passing through sieve 1, were here called fine fractions. The particle size distribution influence characteristics of the final product as smaller particles takes up more water and have a higher influence of viscosity development during mixing and blending in the dough preparation step (Mbaeyi-Nwaoha & Uchendu, 2016). Also, coarse particles can reduce formation of air pockets thus counteract the formation of an airy and expanded breakfast cereal as they may break and disrupt the matrix. Visualized in Figure 31 is the ratios of fine and coarse fractions for the different BSG varieties. The BSG from Dugges contains a larger fraction of coarse particles compared to Senson and Peroni. Peroni contains less coarse particles and has the finest particle size distribution among the BSG. This is most likely due to a more efficient milling procedure carried out for those samples.

Table 8. Particle size distributions for the BSG from the different breweries.

Sieve nr.	Sieving size [µm]	Dugges	Peroni	Senson
5	>1250	2,68	0,03	0,23
4	710–1250	23,39	0,17	4,71
3	500–710	19,19	0,80	14,57
2	250–500	30,08	65,46	62,87
1	<250	24,67	33,28	18,25

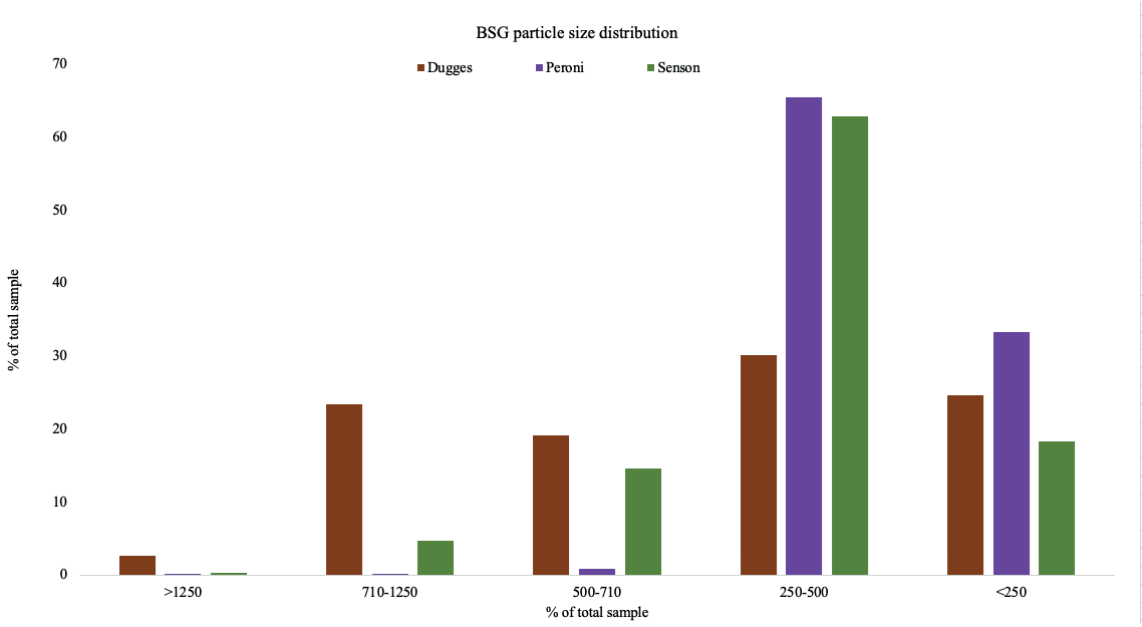


Figure 30. Particle size distributions for the BSG from the different breweries

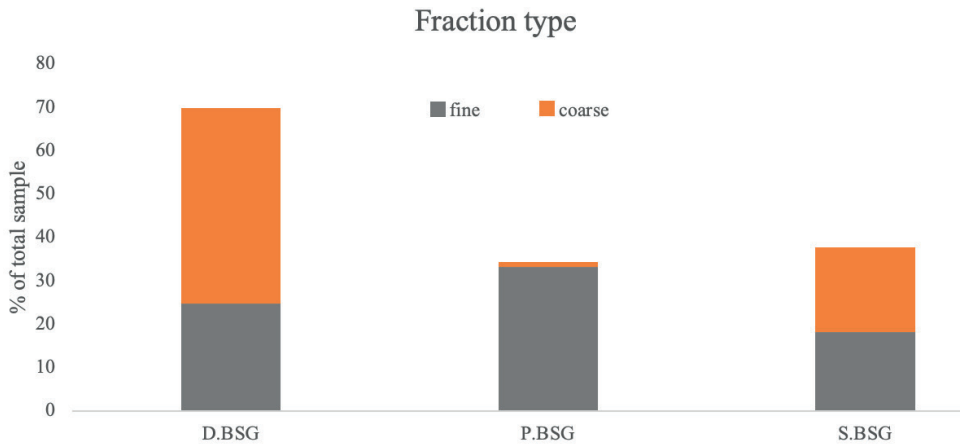


Figure 31. Coarse and fine fractions for the different BSG .

3.5 Sensory analysis

Sensory profile

To obtain an overall visualisation of the sensory profile for each breakfast cereal their average intensity ratings for the attributes evaluated were displayed in radar charts of their dry and wet characteristics, respectively (Figure 32 and Figure 33).

The centre of the coordinate diagram (0,0) in Figure 32 and 33 corresponds to no perceived attribute regarding each descriptor listed and ranging to the maximal intensity of 5,0 at the end of the axis. The sensory profiles appear similar for the prototypes except for the reference without BSG (Rye ref) that deviates profoundly with respect to expansion and crispiness for the dry attributes and crispiness and coarse mouthfeel for its bowl-life characteristics.

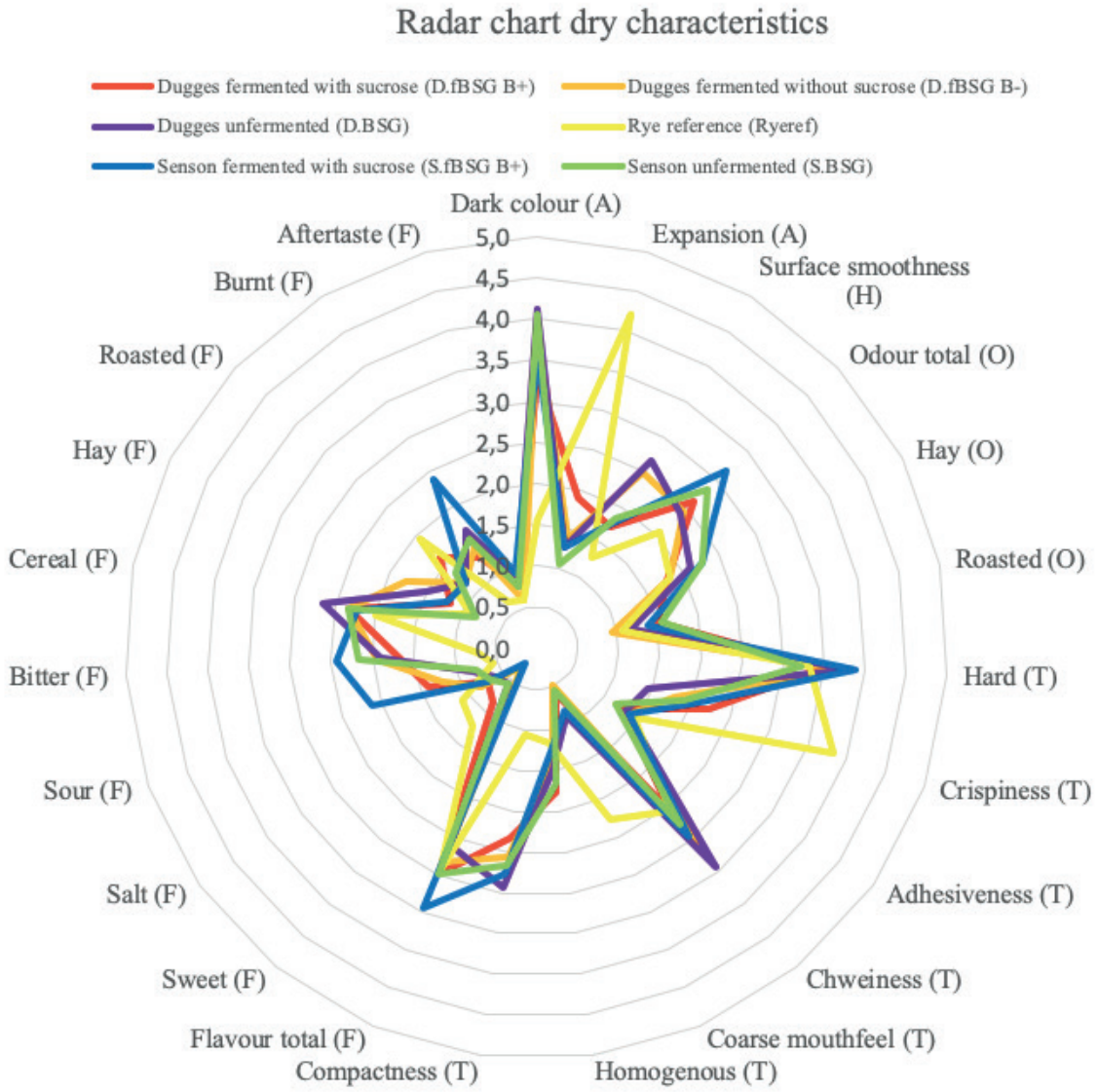


Figure 32. Radar chart dry characteristics for all samples evaluated.

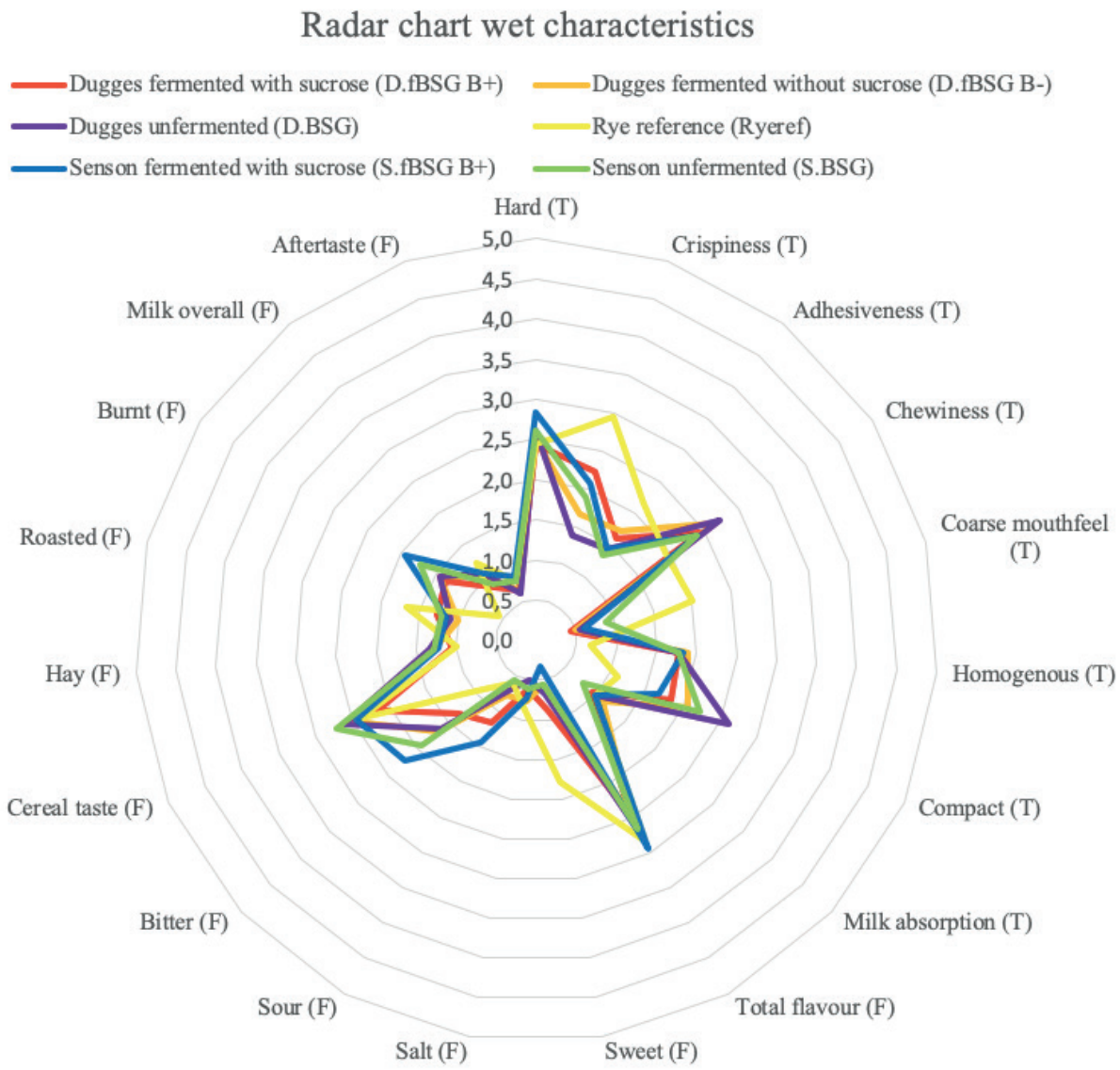


Figure 33. Radar chart bowl-life for all samples evaluated.

ANOVA

A mixed model two-way analysis of variance (ANOVA) with an interaction term between the two factors was conducted on the whole data set to analyse which attributes that differed between the products. The factors used were samples and panellists. The hypothesis to be tested was *H0: βjk=0*, which means no product effect. In the ANOVA used this hypothesis was divided into k separated hypothesis, one for each attribute tested. Tukey's honesty significant difference test (HSD) was used with a p-value limit of 0,05 to test the products based on the factors in the model (Equation 2). Non-significant attributes were eliminated from further analysis.

The results of the ANOVA of the complete data set is displayed in Table 9. It revealed that 20 out of 45 attributes evaluated were significantly different ($p<0,05$) between the breakfast cereal prototypes tested in the sensory analysis.

Based on the significant attributes obtained, odour seemed to be less discriminant for the samples evaluated. Appearance, on the other hand, seemed to be a discriminant factor but this is most likely due to the deviating appearance of the reference (Rye ref) with respect to colour and expansion. Textural attributes such as crispiness, coarse mouthfeel and compactness did not differ significantly between the prototypes of interest but differed compared to the reference.

Flavour attributes displayed some differences between the products, but no clear pattern could be interpreted as the tendency observed differed between the BSG from different breweries. The fermented prototype from Dugges (D.B+) was both sweeter and less bitter compared to its non-fermented control (D.BSG) as well as its EPS-negative control (D.B-). Whereas for the Senson prototypes, the fermented sample was slightly more bitter and less sweet compared to the control.

All samples containing BSG were less sweet and more bitter than Rye ref. Expansion, dark colour and crispiness were found to be the most influential attributes to differentiate between the breakfast cereals with respect to their p-values (Table 9).

Table 9. Significant attributes for the prototypes tested based on a mixed model two-way ANOVA. (A)=appearance, (O)=odour, (T)=texture and (F)=flavour.

Sensory attributes	**S.B+**	**D.BSG**	**D. B-**	**S.BSG**	**Ryeref**	**D.B+**	**p-values**
Dry							
Dark colour (A)	3,556 bc	4,111 a	3,889 ab	4,056 ab	1,556 d	3,333 c	1,27E-25
Expansion (A)	1,278 c	1,278 c	1,389 bc	1,056 c	4,222 a	1,889 b	7,69E-31
Surface smoothness (H)	1,778 c	2,667 a	2,500 ab	1,833 bc	1,278 c	1,722 c	4,80E-07
Odour total (O)	3,167 a	2,389 bc	2,444 abc	2,833 ab	2,056 c	2,611abc	8,56E-04
Crispiness (T)	2,000 b	1,444 b	1,778 b	1,889 b	3,833 a	2,222 b	1,23E-11
Coarse mouthfeel (T)	0,833 b	0,889 b	0,500 b	0,556 b	2,278 a	0,611 b	1,88E-06
Compactness (T)	2,778 a	2,944 a	2,556 a	2,667 a	1,056 b	2,333 a	5,58E-09
Sweet (F)	0,222 c	0,556 bc	0,278 bc	0,556 bc	1,222 a	0,833 ab	4,28E-05
Salt (F)	0,722 ab	0,611 b	0,833 ab	0,667 ab	1,111 a	0,722 ab	3,16E-02
Sour (F)	2,111 a	0,833 bc	1,222 bc	0,778 bc	0,556 c	1,389 ab	2,56E-06
Bitter (F)	2,444 a	1,889 a	1,944 a	2,167 a	0,722 b	1,667 a	1,99E-04
Roasted (F)	1,167 a	1,167 a	1,167 a	1,333 a	1,944 a	1,611 a	3,57E-02
Burnt (F)	2,389 a	1,667 ab	1,444 abc	1,556 abc	0,667 c	1,278 bc	1,15E-04
Wet							
Crispiness (T)	2,056 bc	1,389 c	1,667 bc	1,889 bc	2,944 a	2,222 b	3,86E-08
Coarse mouthfeel (T)	0,611 b	0,556 b	0,500 b	0,889 b	2,000 a	0,444 b	1,88E-06
Homogenous (T)	1,833 a	1,778 a	1,889 a	1,778 a	0,667 b	1,778 a	2,00E-04
Compact (T)	1,667 ab	2,611 a	2,056 ab	2,222 a	1,111 b	1,833 ab	8,84E-04
Sweet (F)	0,333 b	0,667 b	0,556 b	0,556 b	1,778 a	0,889 b	2,72E-08
Sour (F)	1,444 a	0,722 ab	0,778 ab	0,556 b	0,611 b	1,167 ab	3,86E-03
Bitter (F)	2,222 a	1,611 abc	1,667 abc	1,944 ab	0,889 c	1,333 bc	1,99E-04
Burnt (F)	1,944 a	1,444 a	1,444 a	1,722 a	0,556 b	1,333 ab	6,46E-05

a-c: Average values of each sensory attribute followed by the same letters are not significantly different ($p<0,05$)

PCA

To describe the systematic variation of the data principal component analysis (PCA) was performed for the dry characteristics and the wet characteristics, separately. No clear separation between the samples, visualized as dots, were seen in the score plots of neither dry nor wet characteristics in Figure 34 and 36.

Taken both scores and loading into account biplots of dry and wet characteristics are displayed in Figure 35 and 37. Most samples clustered together except the rye reference that showed strong deviations with respect to textural attributes like expansion, crispiness and coarse mouthfeel compared to the other.

Dry characteristics

For the dry PCA (Figure 35) the first two principal components, PC1 and PC2, accounted for 49,9% in total of the variance of the data. 33,8% of the variation between the samples is described by PC1. This component was heavily loaded on attributes like dark colour, compactness, bitter and burnt flavour along the positive x-axis. The second principal component (PC2), explaining 16,1% of the variance, was correlated with the flavour attributes roasted, salt and sweet.

Descriptors found to be positively correlated among the attributes for the dry breakfast cereals were surface smoothness and compactness. Flavour attributes like bitter and burnt were strongly correlated, most likely due to difficulties in distinguish between them. Textural attributes like crispiness and expansion were found positively correlated which was seen also in the instrumental texture analysis (3.3.1).

No obvious differences were revealed with respect to the influence of dextran. However, patterns seen in the biplot (Figure 35) implies that the dextran-negative sample (D.B-) cluster more like the unfermented control (D.BSG) than the dextran-positive sample (D.B+). Thus, fermentation with added sucrose and hence probably more dextran produced seems to have a positive influence on attributes like crispiness, expansion, sweetness, coarse mouthfeel and roasted compared to the dextran-negative control with similar BSG.

The increased sweetness and less bitterness perceived can be deduced to either the presence of dextran itself or that not all sucrose added in the fermentation has been completely converted by the LAB and hence, some sucrose residues left. The sweetness can mask bitter substances like polyphenols that may become more prominent during fermentation due to degradation of cell walls. This seems to be valid for the prototypes from Dugges.

The fermented prototype from Senson deviated slightly from the other with respect to the flavour attributes sour and bitter/burnt according to the scores and loadings plots in Figure 34.

A hypothesis is that this discrepancy can be related to the difference detected in the AX analysis of the immunolabelled cross-sections (3.1.3) where the most distinct difference between fermented and control in terms of fragmentation was seen for Senson. Release of phenolic substances bound to components in the cell walls due to degradation may influence the flavour as many polyphenols are recognized to be bitter.

Further, the smaller particle size observed for BSG from Senson compared to Dugges promotes more interactions between LAB and components in the BSG thus leading to a more effective fermentation process. The lower pH and higher acidification obtained after fermentation of Senson BSG further strengthens this hypothesis and can explain the increased sourness for these prototypes.

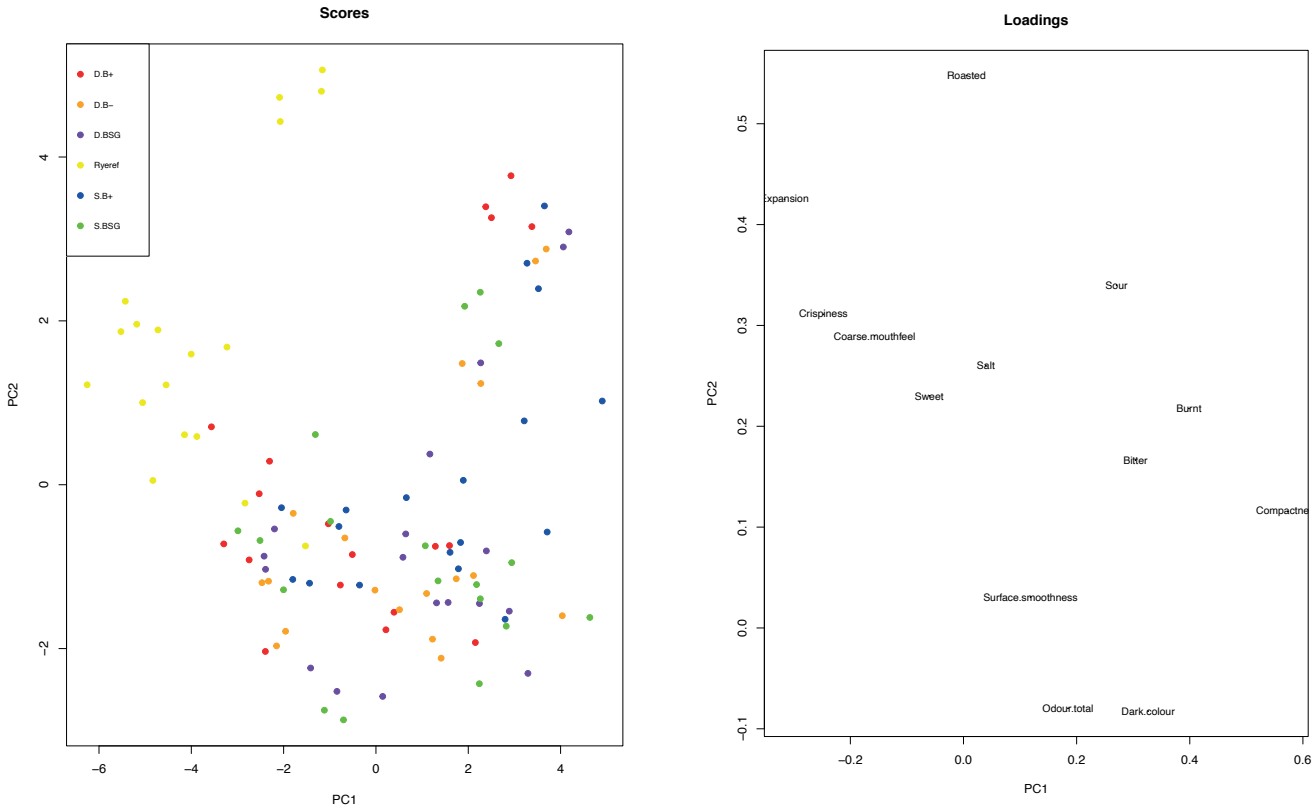


Figure 34. PCA: score and loading plot of dry characteristics.

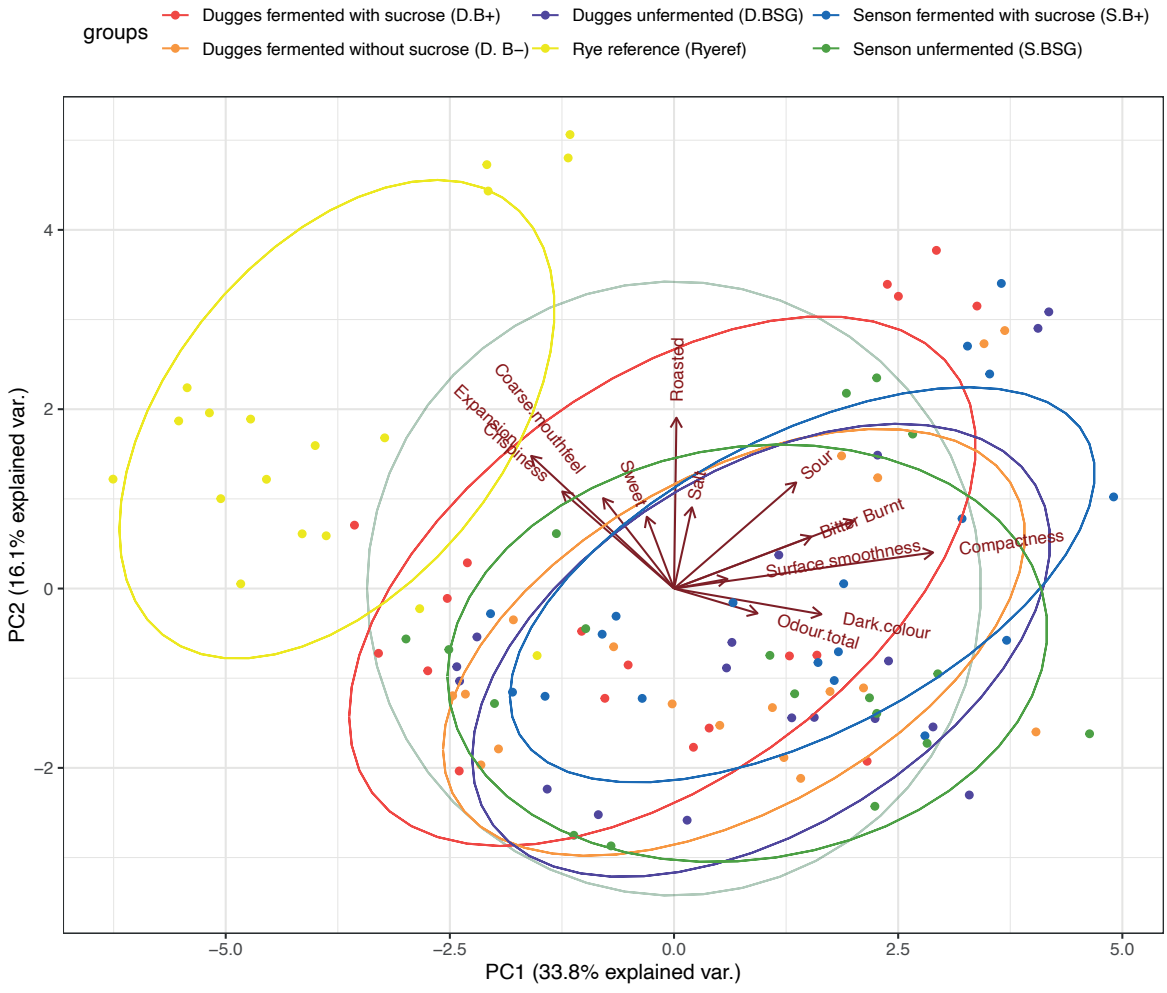


Figure 35. PCA: Biplot of dry characteristics explaining 49,9% of the variance in the data.

Wet characteristics

The PCA of the wet characteristics evaluated after 3 min immersion in milk (3% fat), displayed in Figure 37, explains 61,2% of the variance in the data. PC1 that accounts for 41,2% of the explained variance corresponds to the descriptors compactness and burnt.

20,0% of the variance was explained by the second principal component (PC2) that was loaded with attributes like bitterness, sourness and homogenous texture.

The same overall pattern as for dry characteristics was also reflected in the PCA of the wet descriptors. Most samples overlapped each other except for the rye reference that was perceived as crispier, coarser mouthfeel and less homogenous compared to the bowl-life of the BSG prototypes.

The PCA analysis, both for dry and wet samples, was not enough to completely discriminate between the different bioprocessing treatments carried out as most prototypes except the rye reference clustered very similar. However, the PCA showed that the most important attributes to differentiate the prototypes from the rye reference without BSG were expansion, crispiness, coarse mouthfeel and homogeneity.

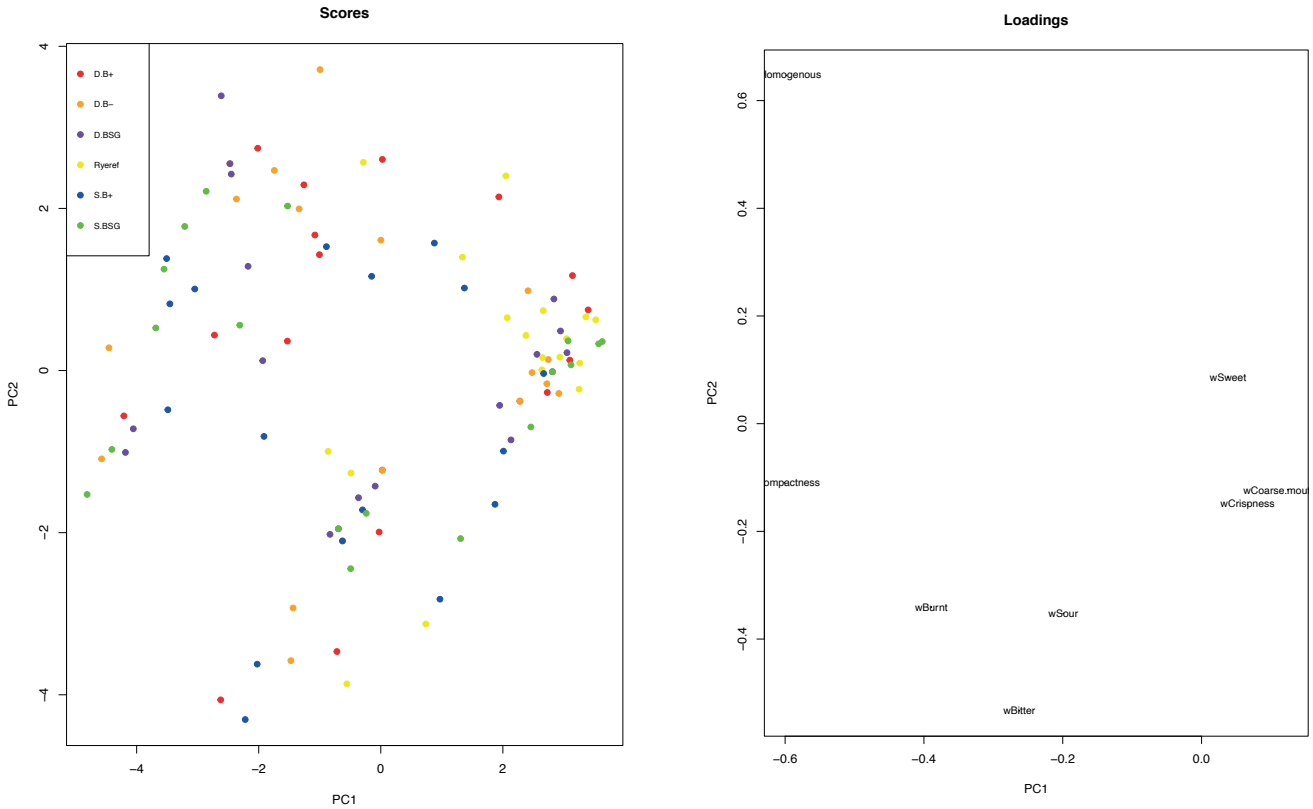


Figure 36. PCA: score and loading plot of wet characteristics.

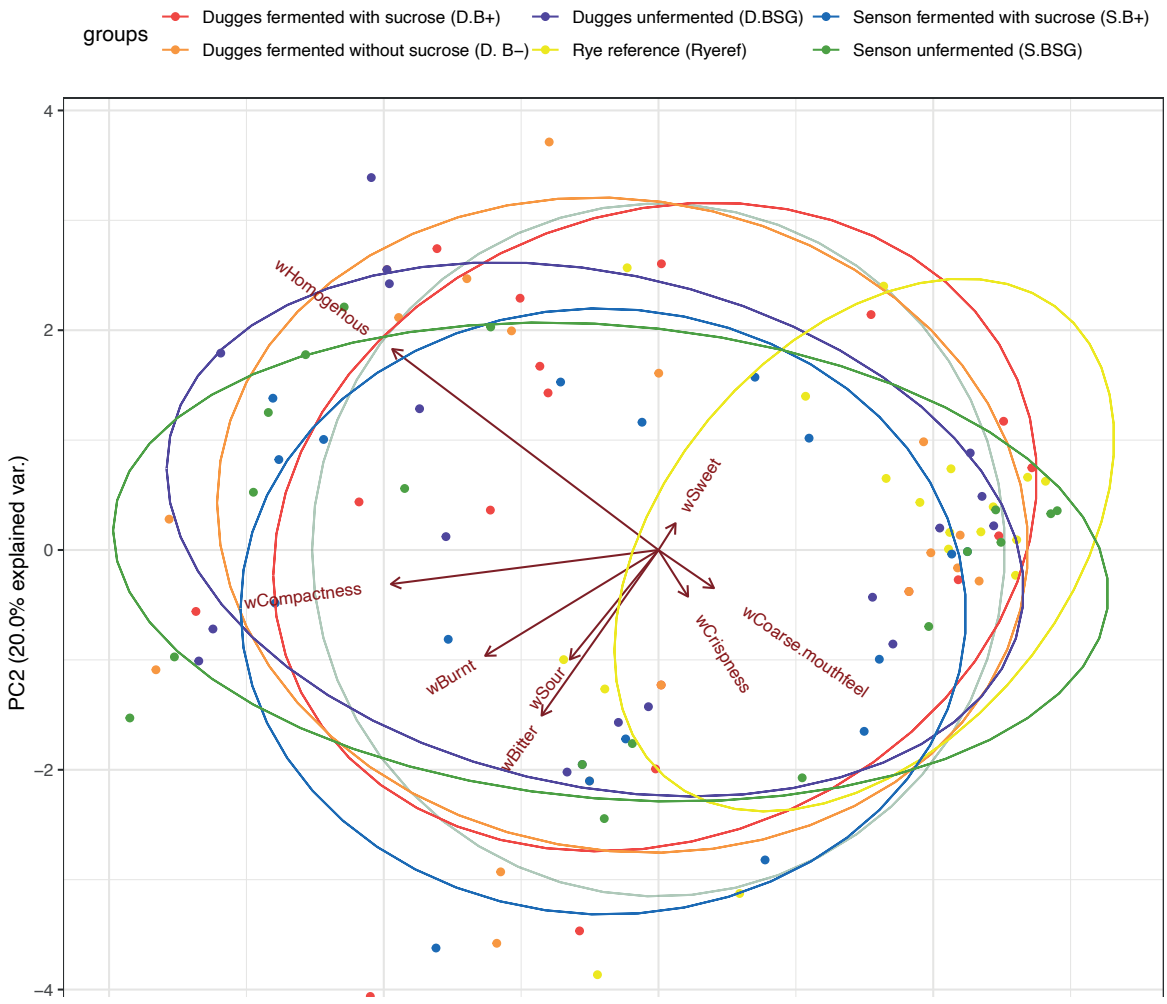


Figure 37. PCA: Biplot of wet characteristics explaining 61,2% of the variance in the data.

4. Evaluation of methodology

The methods used for the microstructural analysis were suitable for qualitative visualization of the overall morphology and specific structures in the matrix of the samples. However, for a more quantitative comparison of the pores and fibre structures a quantitative image analysis technique could have been applied and coupled to the confocal microscopy analysis.

Further, to more thoroughly analyse the influence of dextran a chemical analysis approach could have been undertaken by using HPLC coupled to LC-MS/MS in order to investigate the composition and abundance of dextran in the different prototypes.

A set peak threshold could have been implemented to increase the significance of the textural analysis regarding estimation of peak numbers for comparison of crispiness between the samples analysed.

In terms of the sensory analysis the RATA method was assumed to yield enought information for the aim. Quantitative descriptive analysis (QDA) may have been able to display more subtle variations between the different bioprocessing methods, more time-consuming and expensive though.

5. CONCLUSION

A combination of various analysis methods has been applied to characterize the microstructure, texture and sensory profile of the final breakfast cereal prototypes. The aim was to reveal the impact of incorporation of bioprocessed BSG and the polysaccharide dextran to establish an overall understanding of the correlation between bioprocessing and properties of the final product.

The microstructural profiles of the BSG containing prototypes showed increased density and less pores compared to the control without BSG and conventional breakfast cereals. The texture analysis confirmed that the addition of BSG resulted in a hard and compact texture with better retained crispiness after immersion in milk compared to conventional breakfast cereals, hence suitable for slow eaters. The larger fibre structures attributed to BSG were found to have a reinforcing effect on the matrix. The sensory evaluation further showed that incorporation of BSG render attributes like compactness and dark colour.

A dispersed and more fragmentized protein distribution could be seen in the microstructural analysis of the tailored bioprocessing with selected LAB (and enzymes) compared to their respective controls. Further, immunostaining of AX displayed signs of cell wall degradation as AX was seen released from the cell walls in the bioprocessed samples. This can be a sign of increased bioaccessibility of phenolic substances bound to lignocellulosic structures in the cell walls.

For the textural and sensorial evaluations no clear differences were observed regarding the bioprocessing treatments. Differences observed between BSG from the different breweries were associated with variations in particle size distribution and composition. Hence, it was concluded that these factors seemed to have a larger impact on the final product than the bioprocessing itself.

Potential influence of dextran that was observed was a more homogenous outlook of the matrix, increased sweetness perceived and a more affected texture after soaking in milk when comparing the dextran-positive sample to its negative control with BSG from Dugges.

The complexity of extruded breakfast cereals constituting of different ingredients, BSG compositions and bioprocessing techniques applied makes it hard to draw conclusions regarding the influence of individual factors other than general patterns observed. The hypothesis that the bioprocessing would yield an improved structure and enhanced nutritional and sensorial quality of the cereals could not be completely strengthened as the treatments applied did not fully counteract the negative effects of BSG on textural properties like hardness and compactness.

Cereals with decent porosity, crispiness and expansion along with pleasant taste are favoured among consumers. Given the large supply of BSG produced annually and its potential as a nutritious and functional food ingredient further research is needed to find the optimal composition and bioprocessing parameters for breakfast cereals containing BSG.

References

Alam, S. A., Järvinen, J., Kirjoranta, S., Jouppila, K., Poutanen, K., & Sozer, N. (2014). Influence of Particle Size Reduction on Structural and Mechanical Properties of Extruded Rye Bran. Food and Bioprocess Technology, 7(7), 2121–2133. https://doi.org/10.1007/s11947-013-1225-2

Anton, A. A., & Luciano, F. B. (2007). Instrumental texture evaluation of extruded snack foods: A review. *Ciencia y Tecnologia Alimentaria*, *5*(4), 245–251. https://doi.org/10.1080/11358120709487697

Antonini, E., Zara, C., Valentini, L., Gobbi, P., Ninfali, P., & Menotta, M. (2018). Novel insights into pericarp, protein body globoids of aleurone layer, starchy granules of three cereals gained using atomic force microscopy and environmental scanning electronic microscopy. *European Journal of Histochemistry*, *62*(1), 20–27. https://doi.org/10.4081/ejh.2018.2869

Autio, K., & Salmenkallio-Marttila, M. (2001). Light Microscopic Investigations of Cereal Grains, Doughs and Breads. *LWT - Food Science and Technology*, *34*(1), 18–22. https://doi.org/10.1006/fstl.2000.0725

Basic Statistical Concepts for Sensory. (1986).

Cartier, R., Rytz, A., Lecomte, A., Poblete, F., Krystlik, J., Belin, E., & Martin, N. (2006). Sorting procedure as an alternative to quantitative descriptive analysis to obtain a product sensory map. *Food Quality and Preference*, *17*(7–8), 562–571. https://doi.org/10.1016/j.foodqual.2006.03.020

Chanvrier, H., Desbois, F., Perotti, F., Salzmann, C., Chassagne, S., Gumy, J. C., & Blank, I. (2013). Starch-based extruded cereals enriched in fibers: A behavior of composite solid foams. *Carbohydrate Polymers*, *98*(1), 842–853. https://doi.org/10.1016/j.carbpol.2013.07.005

Collins, H. M., Burton, R. A., Topping, D. L., Liao, M. L., Bacic, A., & Fincher, G. B. (2010). Variability in fine structures of noncellulosic cell wall polysaccharides from cereal grains: Potential importance in human health and nutrition. *Cereal Chemistry*, *87*(4), 272–282. https://doi.org/10.1094/CCHEM-87-4-0272

Department of Neuroscience/Ophthalmology, Akademiska sjukhuset, S.-751 85 U. (2006). Generella och specifika histologiska analysmetoder. Retrieved February 13, 2020, from http://www2.neuro.uu.se/mun/mcb/histologi.LINK/introduktion.htm

Dornez, E., Holopainen, U., Cuyvers, S., Poutanen, K., Delcour, J. A., Courtin, C. M., & Nordlund, E. (2011). Study of grain cell wall structures by microscopic analysis with four different staining techniques. *Journal of Cereal Science*. https://doi.org/10.1016/j.jcs.2011.07.004

Eriksson, J., & Eriksson, J. (2012). Barley starch , structure and properties, (362).

Faller', J. F., Faller, J. Y., & Klein, B. P. (2000). *PHYSICAL AND SENSORY CHARACTERISTICS OF EXTRUDED CORN/SOY BREAKFAST CEREALS*.

Fleming, E. E., Ziegler, G. R., & Hayes, J. E. (2015). Check-all-that-apply (CATA), sorting, and polarized sensory positioning (PSP) with astringent stimuli. *Food Quality and Preference*, *45*, 41–49. https://doi.org/10.1016/j.foodqual.2015.05.004

Heiniö, R.-L., Katina, K., Alam, S. A., Sozer, N., & Kock, D. (2015). *Sensory characteristics of wholegrain and bran-rich cereal foods-a review*.

Jääskeläinen, A. S., Holopainen-Mantila, U., Tamminen, T., & Vuorinen, T. (2013). Endosperm and aleurone cell structure in barley and wheat as studied by optical and Raman microscopy. *Journal of Cereal Science*, *57*(3), 543–550. https://doi.org/10.1016/j.jcs.2013.02.007

Jekle, M., & Becker, T. (2015). Wheat Dough Microstructure: The Relation Between Visual Structure and Mechanical Behavior. *Critical Reviews in Food Science and Nutrition*, *55*(3), 369–382. https://doi.org/10.1080/10408398.2012.656476

John D. Bancroft, K. S. (2012). *Theory and Practice of Histological Techniques* (7th ed.). Churchill Livingstone.

Kajala, I., Mäkelä, J., Coda, R., Shukla, S., Shi, Q., Maina, N. H., … Katina, K. (2016). Rye bran as fermentation matrix boosts in situ dextran production by Weissella confusa compared to wheat bran. *Applied Microbiology and Biotechnology*, *100*(8). https://doi.org/10.1007/s00253-015-7189-6

Ktenioudaki, A., Alvarez-Jubete, L., Smyth, T.J., Kilcawley, K., & Gallagher, E. (2015). Application of bioprocessing techniques for brewer's spent grain breads. *Food res*. Int. 73, 107-116. doi: 10.1016/j.foodres.2015.03.008

Kälviäinen, N., Salovaara, H., & Tourila, H. (2002). Sensory attributes and preference mapping of muesli oat flakes. *Journal of Food Science*, *67*(1), 455–460. https://doi.org/10.1111/j.1365-2621.2002.tb11428.x

Karlsen, A. M., Aaby, K., Sivertsen, H., Baardseth, P., & Ellekjær, M. R. (1999). Instrumental and sensory analysis of fresh Norwegian and imported apples. *Food Quality and Preference*, *10*(4–5), 305–314. https://doi.org/10.1016/s0950-3293(99)00030-0

Lenfant, F., Loret, C., Pineau, N., Hartmann, C., & Martin, N. (2009). Perception of oral food breakdown. The concept of sensory trajectory. *Appetite*, *52*(3), 659–667. https://doi.org/10.1016/j.appet.2009.03.003

Luciano, G., & Næs, T. (2009). Interpreting sensory data by combining principal component analysis and analysis of variance. *Food Quality and Preference*, *20*(3), 167–175. https://doi.org/10.1016/j.foodqual.2008.08.003

Lynch, K. M., Steffen, E. J., & Arendt, E. K. (2016a). Brewers' spent grain: a review with an emphasis on food and health. *Journal of the Institute of Brewing*. John Wiley and Sons Inc. https://doi.org/10.1002/jib.363

Lynch, K. M., Steffen, E. J., & Arendt, E. K. (2016b). Brewers' spent grain: a review with an emphasis on food and health. *Journal of the Institute of Brewing*, *122*(4), 553–568. https://doi.org/10.1002/jib.363

Mauko, A., Muck, T., Mirtič, B., Mladenovič, A., & Kreft, M. (2009). Use of confocal laser scanning microscopy (CLSM) for the characterization of porosity in marble. *Materials Characterization*, *60*(7), 603–609. https://doi.org/10.1016/j.matchar.2009.01.008

Mbaeyi-Nwaoha, I. E., & Uchendu, N. O. (2016). Production and evaluation of breakfast cereals from blends of acha and fermented soybean paste (okara). *Journal of Food Science and Technology*, *53*(1), 50–70. https://doi.org/10.1007/s13197-015-2032-8

McCarthy, A. L., O'Callaghan, Y. C., Piggott, C. O., FitzGerald, R. J., & O'Brien, N. M. (2013). Brewers' spent grain; Bioactivity of phenolic component, its role in animal nutrition and potential for incorporation in functional foods: A review. In *Proceedings of the Nutrition Society* (Vol. 72, pp. 117–125). https://doi.org/10.1017/S0029665112002820

McCartney, L., Marcus, S. E., & Knox, J. P. (2005). Monoclonal antibodies to plant cell wall xylans and arabinoxylans. *Journal of Histochemistry and Cytochemistry*, *53*(4), 543–546. https://doi.org/10.1369/jhc.4Bniou6578.2005

Microscopy, L., & Carlsson, K. (n.d.). *Biomedical & X-ray Physics Kjell Carlsson Physics of Biomedical Microscopy*.

Mussatto, S. I., Dragone, G., & Roberto, I. C. (2006, January). Brewers' spent grain: Generation, characteristics and potential applications. *Journal of Cereal Science*. https://doi.org/10.1016/j.jcs.2005.06.001

Nikinmaa, M., Mattila, O., Holopainen-Mantila, U., Heiniö, R. L., & Nordlund, E. (2019). Impact of lactic acid bacteria starter cultures and hydrolytic enzymes on the characteristics of wholegrain crackers. *Journal of Cereal Science*, *88*(April), 1–8. https://doi.org/10.1016/j.jcs.2019.04.016

Oliveira, L. C., Alencar, N. M. M., & Steel, C. J. (2018). Improvement of sensorial and technological characteristics of extruded breakfast cereals enriched with whole grain wheat flour and jabuticaba (Myrciaria cauliflora) peel. *LWT - Food Science and Technology*, *90*, 207–214. https://doi.org/10.1016/j.lwt.2017.12.017

Ozilgen, S., & Bucak, S. (2018). Functional Biopolymers in Food Manufacturing. In *Biopolymers for Food Design* (pp. 157–189). Elsevier Inc. https://doi.org/10.1016/B978-0-12-811449-0.00006-2

Part C Food Structure Analysis, Characterisation, and Modelling. (n.d.). Retrieved from http://ebookcentral.proquest.com/lib/chalmers/detail.action?docID=5976904.

Poutanen, K., Flander, L., & Katina, K. (2009). Sourdough and cereal fermentation in a nutritional perspective. *Food Microbiology*, *26*(7), 693–699. https://doi.org/10.1016/j.fm.2009.07.011

Pozen, M. A. (1934). Enzymes in Brewing. Industrial and Engineering Chemistry, 26(11), 1127–1133. https://doi.org/10.1021/ie50299a002

Qin, F., Johansen, A. Z., & Mussatto, S. I. (2018). Evaluation of different pretreatment strategies for protein extraction from brewer's spent grains. *Industrial Crops and Products*, *125*, 443–453. https://doi.org/10.1016/j.indcrop.2018.09.017

Robin, F., & Palzer, S. (2015). Texture of breakfast cereals and extruded products. In *Modifying Food Texture: Novel Ingredients and Processing Techniques* (pp. 203–235). Elsevier. https://doi.org/10.1016/B978-1-78242-333-1.00010-3

Romano, R. (2007). The use of analysis of variance and

three-way factor analysis methods for studying the quality of a sensory panel. *Seven*, 1–4.

Roos, Y. H. (1995). Physical State and Molecular Mobility. *Phase Transitions in Foods*, 19–48. https://doi.org/10.1016/b978-012595340-5/50002-x

Sacchetti, G., Pittia, P., & Pinnavaia, G. G. (2005). The effect of extrusion temperature and drying-tempering on both the kinetics of hydration and the textural changes in extruded ready-to-eat breakfast cereals during soaking in semi-skimmed milk. *International Journal of Food Science & Technology*, *40*(6), 655–663. https://doi.org/10.1111/j.1365-2621.2005.00976.x

Sakhare, S. D., Inamdar, A. A., Soumya, C., Indrani, D., & Rao, G. V. (2014). Effect of flour particle size on microstructural, rheological and physico-sensory characteristics of bread and south Indian parotta. *Journal of Food Science and Technology*, *51*(12), 4108–4113. https://doi.org/10.1007/s13197-013-0939-5

Salawu, S. O., Bester, M. J., & Duodu, K. G. (2014). Phenolic composition and bioactive properties of cell wall preparations and whole grains of selected cereals and legumes. *Journal of Food Biochemistry*, *38*(1), 62–72. https://doi.org/10.1111/jfbc.12026

Scientific, T. F. (n.d.). Immunolabeling. Retrieved from https://www.thermofisher.com/se/en/home/life-science/cell-analysis/cell-analysis-learning-center/molecular-probes-school-of-fluorescence/imaging-basics/labeling-your-samples/immunolabeling.html

Stojceska, V., Ainsworth, P., Plunkett, A., & Ibanoğlu, S. (2008). The recycling of brewer's processing by-product into ready-to-eat snacks using extrusion technology. *Journal of Cereal Science*, *47*(3), 469–479. https://doi.org/10.1016/j.jcs.2007.05.016

The University of Oklahoma (Preston Larson, S. D. R. (2019). PREPARATION OF BIOLOGICAL SPECIMENS. Retrieved February 13, 2020, from http://www.ou.edu/research/electron/bmz5364/prepare.html

Title, P., Project, Y., Related, I. S., Duration, P., Funding, T. R., & Costs, T. (2020). PROJECT : FUNBREW.

Traill, R. M., Luckman, M. S., Fisk, N. A., & Peng, M. (2019). Application of the Rate-All-That-Apply (RATA) method to differentiate the visual appearance of milk powders using trained sensory panels. *International Dairy Journal*, *97*, 230–237. https://doi.org/10.1016/j.idairyj.2019.05.013

Valles Pamies, B., Roudaut, G., Dacremont, C., Le Meste, M., & Mitchell, J. R. (2000). Understanding the texture of low moisture cereal products: Mechanical and sensory measurements of crispiness. *Journal of the Science of Food and Agriculture*, *80*(11), 1679–1685. https://doi.org/10.1002/1097-0010(20000901)80:11<1679::AID-JSFA697>3.0.CO;2-P

Vázquez-Gutiérrez, J. L., & Langton, M. (2015, February 1). Current potential and limitations of immunolabeling in cereal grain research. *Trends in Food Science and Technology*. Elsevier Ltd. https://doi.org/10.1016/j.tifs.2014.10.002

Verheugen, G. (2005). European commission. *Pharmaceuticals Policy and Law*, *6*(October), 1–2. https://doi.org/10.4324/9781849776110-28

Verni, M., Pontonio, E., Krona, A., Jacob, S., Pinto, D., & Rinaldi, F. et al. (2020). Bioprocessing of brewer's spent grain enhances its antioxidant activity: Characterisation of phenolic compounds and bioactive peptides. *Frontiers In Microbiology*, 11. doi: 10.3389/fmicb.2020.01831

Wang, Y., Sorvali, P., Laitila, A., Maina, N. H., Coda, R., & Katina, K. (2018). Dextran produced in situ as a tool to improve the quality of wheat-faba bean composite bread. *Food Hydrocolloids*, *84*. https://doi.org/10.1016/j.foodhyd.2018.05.042

Wójtowicz, A., Mitrus, M., Oniszczuk, T., Mościcki, L., Kręcisz, M., & Oniszczuk, A. (2015). Selected Physical Properties, Texture and Sensory Characteristics of Extruded Breakfast Cereals based on Wholegrain Wheat Flour. *Agriculture and Agricultural Science Procedia*, *7*, 301–308. https://doi.org/10.1016/j.aaspro.2015.12.051

Appendix A

Protocol for plastic embedding with Technovit 7100 (T7100) is displayed in table 1.

Step	Solution	Ratio	Time	Repeats	Temperature
1	Ethanol (90%)	1	15min	2	RT
2	Ethanol (96%)	1	20min	2	RT
3	Ethanol (100%)	1	30min	2	RT
4	T7100: Ethanol (100%)	1:1	90min	1	RT
5	T7100: Ethanol (100%)	2:1	24h	1	RT

Protocol for plastic embedding with Technovit 8100 (T8100) is displayed in table 2.

Step	Solution	Time	Temperature (°C)
1	Acetone (100%)	2min	4
2	Acetone (100%)	5min	4
3	Acetone (100%)	5min	4
4	Acetone (100%)	5min	4
5	Acetone (100%)	10min	4
6	Acetone (100%)	10min	4
7	Technovit H8100 with Hardener I	30min	4
8	Technovit H8100 with Hardener I	1h	4
9	Technovit H8100 with Hardener I	1h	4
10	Technovit H8100 with Hardener I	24h	4
11	Technovit H8100 with Hardener I	24h	4
12	Technovit H8100 with Hardener I	24h	4
13	Technovit H8100 with Hardener I	30h	4