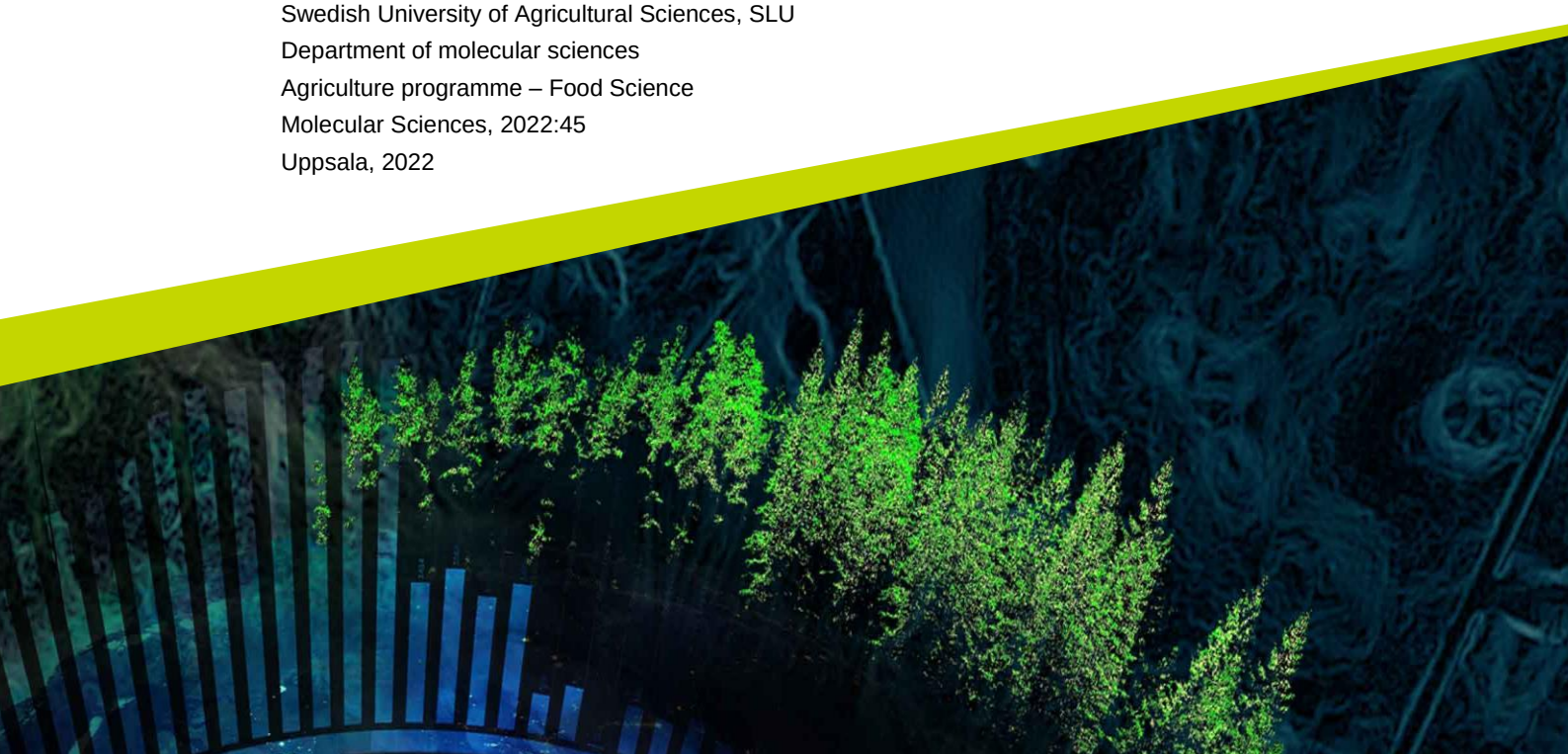
Protein content in Brewer's spent grain and its characteristics

Upcycling spent grain from food waste to protein source

Moa Rönnhagen

Independent project • 30 hp
Swedish University of Agricultural Sciences, SLU
Department of molecular sciences
Agriculture programme – Food Science
Molecular Sciences, 2022:45
Uppsala, 2022



Protein content in Brewer's spent grain and its characteristics - Upcycling spent grain from food waste to protein source

Proteininnehåll hos drav och dess egenskaper
– Upcycling av drav från livsmedelsavfall till proteinkälla.

Moa Rönnhagen

Supervisor:	**Henrik Hansson, Swedish University of Agricultural Sciences, Department of molecular sciences**
Examiner:	Maud Langton, Swedish University of Agricultural Sciences, Department of molecular sciences

Credits:	30 hp
Level:	Advanced level, A2E
Course title:	Master Thesis in Food Science
Course code:	EX0877
Programme/education:	Agriculture programme – Food Science
Course coordinating dept:	Department of molecular sciences
Place of publication:	Uppsala
Year of publication:	2022
Title of Series:	Molecular Sciences
Part number:	2022:45
Keywords:	*Barley,* food waste, *brewers' spent grain, beer, protein, extraction*, characterization, future food

Swedish University of Agricultural Sciences
Faculty of Natural Resources and Agricultural Sciences Department of Molecular Sciences

Publishing and archiving

Approved students' theses at SLU are published electronically. As a student, you have the copyright to your own work and need to approve the electronic publishing. If you check the box for **YES**, the full text (pdf file) and metadata will be visible and searchable online. If you check the box for **NO**, only the metadata and the abstract will be visible and searchable online. Nevertheless, when the document is uploaded it will still be archived as a digital file. If you are more than one author, the checked box will be applied to all authors. Read about SLU's publishing agreement here:

- https://www.slu.se/en/subweb/library/publish-and-analyse/register-and-publish/agreement-for-publishing/.

☒ YES, I/we hereby give permission to publish the present thesis in accordance with the SLU agreement regarding the transfer of the right to publish a work.

☐ NO, I/we do not give permission to publish the present work. The work will still be archived and its metadata and abstract will be visible and searchable.

Abstract

The United Nations have adopted sustainable development goals (SDGs). Within these SDGs, food waste management and waste valorization, an increase in value, is found. For these reasons, it was of interest to study Brewers' spent grain, a residual product from beer production. In 2020 beer was one of the largest categories among the production of alcoholic beverages and Brewers' Spent grain (BSG) was one of the largest sources of waste in beer production.

This study aimed to extract protein from two variants of BSG, dried at different temperatures, and to study some of the protein characteristics. The protein extraction was an alkali-soluble and acid precipitation and was performed in variations. The extraction was most successful when using an alkaline concentration of 55 mM (NaOH) and a pH of 3.8 when isoelectric precipitation was performed. Concentration measurements of the proteins in the precipitates were made through Bradford analysis and for two samples using Kjeldahl analysis. At the most, the concentrations of protein showed to be 53.50% for the BSG dried at 50-60°C, results obtained from a Kjeldahl analysis.

For the characterization, SDS-gels, gel filtration, foaming, gelation, and protein nanofibrilation (PNF) was carried out. The results from the SDS-gel and gel filtration showed indications of protein (or complex) of sizes > 200 kDa. The obtained proteins showed abilities to form gel and foam, but the stability and ability were questionable as the precipitates were not only pure protein. For the same reason, the results of the ability to form PNFs were difficult to draw any conclusions from.

In conclusion protein from the BSG in question was able to be extracted. The precipitates showed the ability to form gel and foams, with varying desirable characteristics. Judging by the protein concentrations obtained in the precipitates and total protein content in the BSG, the use of protein as a valorization may not be the answer.

Keywords: Barley, food waste, *brewers' spent grain, beer, protein, extraction*, characterization, future food

Sammanfattning

FN har antagit mål för hållbar utveckling. Inom dessa globala hållbarhetsmål hittas ämnen som matsvinnshantering och värdeökning av avfall. I och med dessa mål var det av intresse att studera drav, en restprodukt från ölproduktion. År 2020 var öl en av de största kategorierna inom produktion av alkoholdrycker och drav en av de största källorna till avfall i ölproduktionen.

Denna studie syftade till att extrahera protein från två varianter av BSG, torkat vid olika temperaturer och att studera några av proteinets egenskaper. Proteinextraktionen var en alkalilöslig och sur utfällning, som utfördes i variationer. Extraktionen var mest lyckad när man använde en alkalisk koncentration om 55 mM (NaOH) och ett pH på 3,8 när isoelektrisk utfällning utfördes. Proteinkoncentrationerna i utfällningarna bestämdes med hjälp av en Bradford-analys och för två prover med användning av en Kjeldahl-analys. Som mest var koncentrationen av protein 53,50 % för dravet torkat vid 50-60°C och framtaget genom Kjeldahl-analys.

För karakteriseringen utfördes SDS-geler, gelfiltrering, skumning, gelning och försök till protein-nanofibriller (PNF). Resultaten från SDS-gelen och gelfiltreringen visade tecken på protein (eller komplex) med storlekar på > 200 kDa. De erhållna proteinerna kunde bilda gel och skum, men stabiliteten och förmågan var tveksam då fällningarna inte bara var rent protein. Av samma anledning var resultaten av förmågan att bilda PNF svåra att dra några slutsatser kring.

Sammanfattningsvis var det möjligt att extrahera protein från dravet i studien. Fällningarna visade egenskaper som att bilda gel och skum, med varierande grad av användbarhet. Att döma av de proteinkoncentrationer som erhållits i utfällningarna och det teoretiska proteininnehållet i dravet, kanske inte användningen av protein som värdeökning är svaret.

Nyckelord: Korn, matavfall, drav, öl, protein, extraktion, karaktärisering och framtida livsmedel.

Table of contents

List of tables

List of figures

Abbreviations

BSG	Brewers’ spent grain
PNF	Protein nanofibrils
AFM	Atomic force microscope
dw	Dry weight

1. Introduction

Beer is a popular drink both to consume and produce in Sweden. In the year 2019 approximately 460,9 million litres of beer was sold (*Bryggerirapporten 2020* 2020). Among producers of alcoholic beverages, beer breweries were the largest category in 2020 (*Dryckes bransch rapporten 2021* 2021). When brewing beer, the largest amount of waste comes from the Brewers' spent grain (BSG). Brewers' spent grain is a residual product from the beer production, originating from steeped malt included in the brewing process (Barth 2013). The area of use for BSG is usually as an energy source via incineration or as animal feed (Mussatto 2014). Potential use of BSG for human consumptions has been studied and work for increased valorisation of BSG has been carried out. Among others, the functional properties and potential use in bread have been studied (Czubaszek et al. 2021; Waters et al. 2012), followed by the application in pasta (Neylon et al. 2021). The interesting properties, possibly useful for a variety of applications in the food industry, are the high-fibre content with arabinoxylan, proteins, polyphenols, lipids, and minerals. The valuable properties together with the availability and low cost, makes BSG into a reasonable resource for further exploration (Mussatto et al. 2006). Furthermore, reducing waste and reuse our resources is a crucial step for the survival of a sustainable earth and human society, known of today.

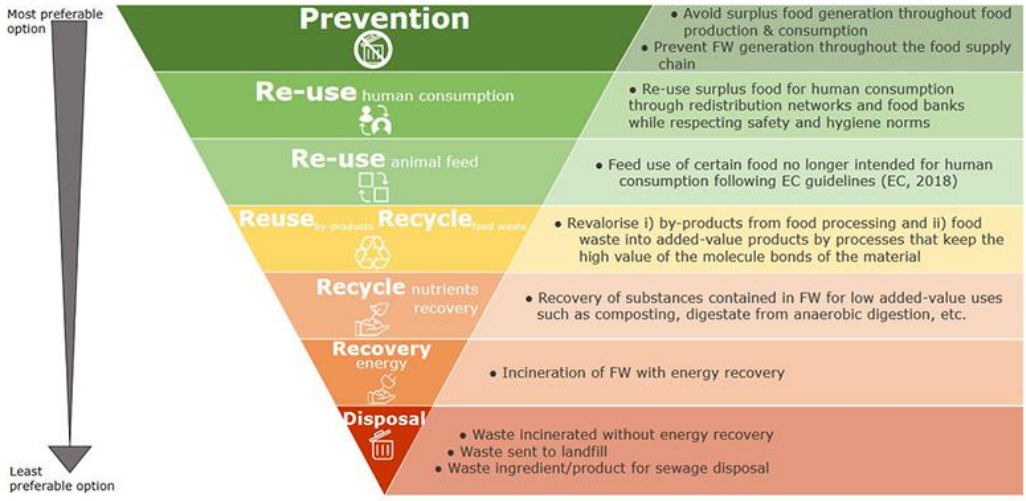


Figure 1. Waste hierarchy, The European Commission's Knowledge Centre for Bioeconomy (2020). Brief on food waste in the European Union.

Responsible consumption and production are one of the sustainable development goals (SDG) within the United Nations (UNDP n.d.). The sustainable development goal 12.5 is about waste management and includes that waste should be reduced, recycled, and prevented. Thus, to the extent that waste has considerably decreased by 2030. It is also stated in SDG 12.3, that food waste should be halved, and that food loses should be reduced from production, post-harvest process and supply chain. Figure 1 shows the waste hierarchy, which visualize the idea of what the aim of waste management surrounds. In the elongated perspective the SDGs was adopted in 2015 by the United Nations to ensure the end poverty, protect the earth and guarantee prosperity for all.

In line with the SDGs, it is therefore important and of interest to valorise the BSG and move the applications upward in the food waste hierarchy.

1.1 Aim

The aim of this master thesis was to extract protein form brewers' spent grain and to characterise the proteins' ability to form foam and gels. The BSG was obtained from a local brewery, Värmdö Bryggeri, in Sweden. The characterisation was made considering their potential application in food for human consumption.

2. Background

2.1 Barley

Barley is a member of the grass family and has the Latin name *Hordeum vulgare*. The cereal is broadly cultivated and commonly used for brewing and as animal feed (Fox 2009). In Sweden year 2021, 22 860 ha of land was used for cultivating winter barley and 255 696 ha for spring barley (Olsson 2021). In relation to other cereals grown in Sweden, barley is the second foremost crop with respect to the distribution of cereal area. Only wheat is cultivated on a larger area. In addition to the different times of sowing, there are two main groups of barley used, which are called six- and two-row barley (Britannica 2020). They are slightly different, besides the quite self-explanatory difference about the number of rows of flowers. The two-row variant is mainly used as malt due to its comparably higher amount of carbohydrates. The six-row barley has higher content of protein and is more commonly used as feed due to this reason.

2.1.1 Structure

The barley grain constitutes of multiple layers and structural parts. Figure 2 shows a schematic picture of the barley seed. Embryo, aleurone layer, endosperm and husk are the main parts of the barley kernel (Fox 2009). The outer layers of the seed are present to protect the interior of the grain (Rosentrater & Evers 2018). Originally, the hull of the barley is tightly attached to the pericarp and hard to separate (Delcour & Hoseney, 2010). Today varieties of barley without hull are present, where the barley loses the hull during threshing. Cellulose is the main component of the husk, together with polyphenols and bitter substances (Arendt & Zannini 2013). Barley grains also contain phenolic acids, polyphenols, catechins reside and proanthocyanidins (Rosentrater & Evers 2018). In total the phenolic compounds are found in a ratio of 0.2 to 0.4% in the barley grain and mainly in the aleurone layer, testa, and hull. In average the hull contributes with 13% of the total grain-weight, with spectrum between 7% and 25% and this depends on growth area, sowing time, two- or six-row variety etc. (Rosentrater & Evers 2018). In Sweden a range between 8-9% has been seen, this as an effect of the distance to the equator. If the latitude of cultivation is closer to the equator the percentage seems to increase (Rosentrater & Evers 2018).

The aleurone layer and region is found closest to the endosperm and is about two or three cells thick (Delcour & Hoseney, 2010). The endosperm is the largest component of the kernel and contains starch and proteins, where the starch is found

in granules and the protein in a surrounding matrix (Fox 2009). One component that is of interest is the ß-d-glucan, one of the major parts of the endosperm cell wall and important for the brewing process (Delcour & Hoseney, 2010). When studying the whole grain, starch is the main component and protein is at a moderate level in the barley grain (Britannica 2020). Approximately 60% of the grain weight is contributed by the starch (Fox 2009). Protein of the barley is mainly found in the endosperm and only a small amount in the hull (Delcour & Hoseney, 2010). The main protein types of barley are hordein and hordenine, which are members of the prolamin and glutelin-group respectively.

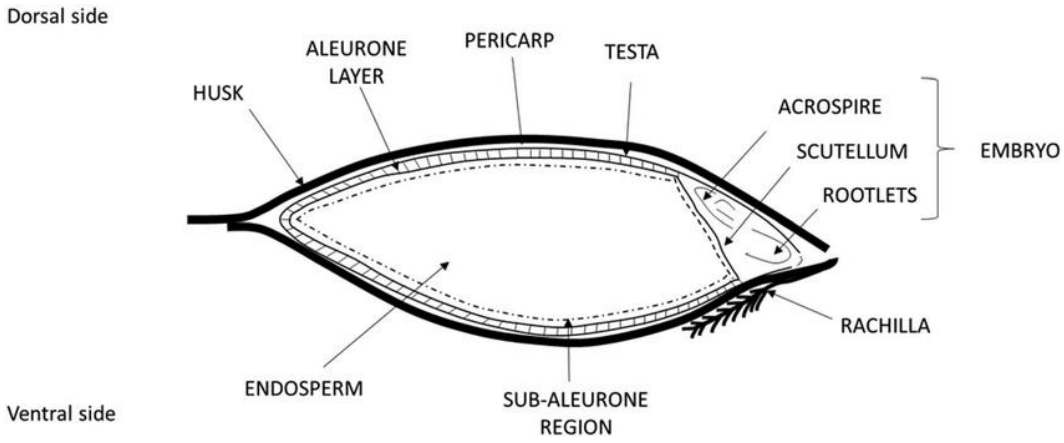


Figure 2. Structure of barley (Filipowska et al. 2021).

2.2 Barley proteins

Barley have different proteins present and depending on the location in the kernel, the amount and type will differentiate (Delcour & Hoseney, 2010). Both storage and structural proteins are found and among them Hordeins and Glutelins are the most common.

2.2.1 Hordeins/prolamins

All proteins are composed of amino acids, and they have different properties that in the end gives different characteristics to the proteins. Hordeins, prolamin proteins, are the major protein variety of barley and is contributing with 30-50% of the total protein (Jonassen et al. 1981; Kirkman et al. 1982; Shewry et al. 1983). In the study made by Shewry et al. (1983), they showed that proline and glutamine were the dominant amino acids in the hordein fraction. Hordeins are present as complex polymorphic blends of polypeptides (Shewry et al. 1988). The hordeins are divided in to different types based on their characteristics and composition of amino acids (Shewry et al. 1983). The types are called A, B, C, D and γ. The B-hordeins are the largest group and with C-hordeins as the second. Only as a small fraction of the total, they are followed by A, D together with γ.

2.2.2 Hordenine/glutelin

Another group of storage proteins are the glutelin, also called hordenine. They are said to contribute with 35-45% of the storage proteins and 10-20% of the total protein (Lásztity 1996). Similarly, to the hordein, glutelin is also rich in proline and glutamine but also glycine and other hydrophobic amino acids. Glutelin has in previous studies also shown to have possibly useful emulsifying properties due to the ratio of polar and non-polar amino acids (Zhao et al. 2011). In order to have the emulsifying properties the glutelin requires exposure to pH 11, down to acidic conditions and then back to neutral pH reported in Wang et al. 2010. This characteristic has its disadvantages and further developments of methods have been done in Zhao et al. 2011.

2.3 Beer production

Beer is generally made of ingredients like water, malted grains, hops and yeast (Barth 2013). The beer production starts with *milling*, where the malt is crushed. Malt is sprouted grains that have been killed by heat. The malted grains could be for example grains of wheat or barley. In this thesis the malt will be referring to barley.

The actual first step of the beer brewing is *mashing*, when hot water and enzymes are allowed to convert starch of the malt into fermentable sugar molecules (Barth 2013). The next step is *wort separation*, where the liquid from the mashing is divided from the brewers' spent grain (BSG), during which some hot water is added (*steeping*) to release all soluble sugars. The liquid phase called sweet wort, contains dextrin and maltose. *Boiling* comes after separation and includes boiling of the sweet wort. Boiling is made to kill unwanted microorganisms and at this stage the hops are usually added to develop flavour and bitterness. After boiling the wort is cooled down, in the *chilling* step. Before fermenting the cooled wort, hops and coagulated proteins are removed, to produce a clear brew. *Fermentation* is the stage of yeast. Depending on the beer, different types of yest is used. The action of the added yeast is to ferment sugar into alcohol. The processes are an anaerobic fermentation, which means it occurs without oxygen. Not only do the yeast provide alcohol, but other reactions also contribute to the development of flavour. When fermentation has occurred, the beer is called green beer. Green beer is exposed to *conditioning* before being finished, as the product known as beer. The conditioning is very beer-specific, meaning that different types of beer will need different conditionings to develop their unique characteristics. Factors as time, filtration, bottles, or use of cask, are differentiating the result of different beers. The finished beer is in the end packed in bottles, cans, or kegs. When packing more carbon dioxide is introduced to give the beer the right characteristics, either with help of yeast or by forced carbonation. The beer is usually pasteurized or exposed to microbial filtration, to remove possible microbes that could spoil the product (Barth 2013).

2.4 Protein characteristics

In this study characteristics are evaluated in a food application prospective. Foaming and gelation have been chosen to be analysed within the frame of this thesis, together with the ability to form protein nanofibrils (PNFs). Dispersion of hydrophobic fluid in a hydrophilic liquid is a form of foam, where air is entrapped in a network of bubbles (Damodaran et al. 2017). The foam is important as it affects the properties of the food product, texture, appearance etc. (Zayas 1997). Gels are usually identified as networks of polymers and particles, when speaking of gels in food (Damodaran et al. 2017). Gels are contributing to structure, texture, stability and thickness of the food (Nazir et al. 2017). Protein nanofibrils are stable polypeptides in a network of hydrogen bonds and a high content of β-sheets (Sipe et al. 2016). Due to their ability to enhance other abilities of characteristics (Akkermans et al., 2008). Therefor the ability to form gel, foam and PNFs are of interest.

3. Material and Method

3.1 Material

For this study Brewer's spent grain (BSG) was obtained from a local brewery, Värmdö Bryggeri, in February 2022. Two types of BSG were used in the experiments, one that had been dried at 100-125°C, called X and another batch dried at 50-60°C that have been called Y. The BSG was delivered dried in boxes of around 2 kg each.

After arrival the BSG (X and Y) was milled by a Retsch ZM200 at speed 18000 rpm, before used in further experiments. The dry matter of the flour was measured by weighing aluminium forms until constant weigh and thereafter together with the flour until constant weight, approximately 24h in 105°C. By measuring the weight before and after heating the dry matter was determined.

3.2 Protein extraction

Protein extraction of Brewer's spent grain was carried out by an alkali-soluble and acid precipitation. The obtained extracts were freeze dried until further testing. Figure 3 outlines the protein extraction schematically. The method of extraction was primarily based from the study done by Suchkov, et al. (1990). In the study by Suchkov, et al. (1990) they looked at pea seeds and broad beans. From the legumes they developed a technique for extraction and isolation of 7s and 11s globulins. In order to modify the method in favour of protein extraction from BSG, the study by Connolly et al. (2013) was used.

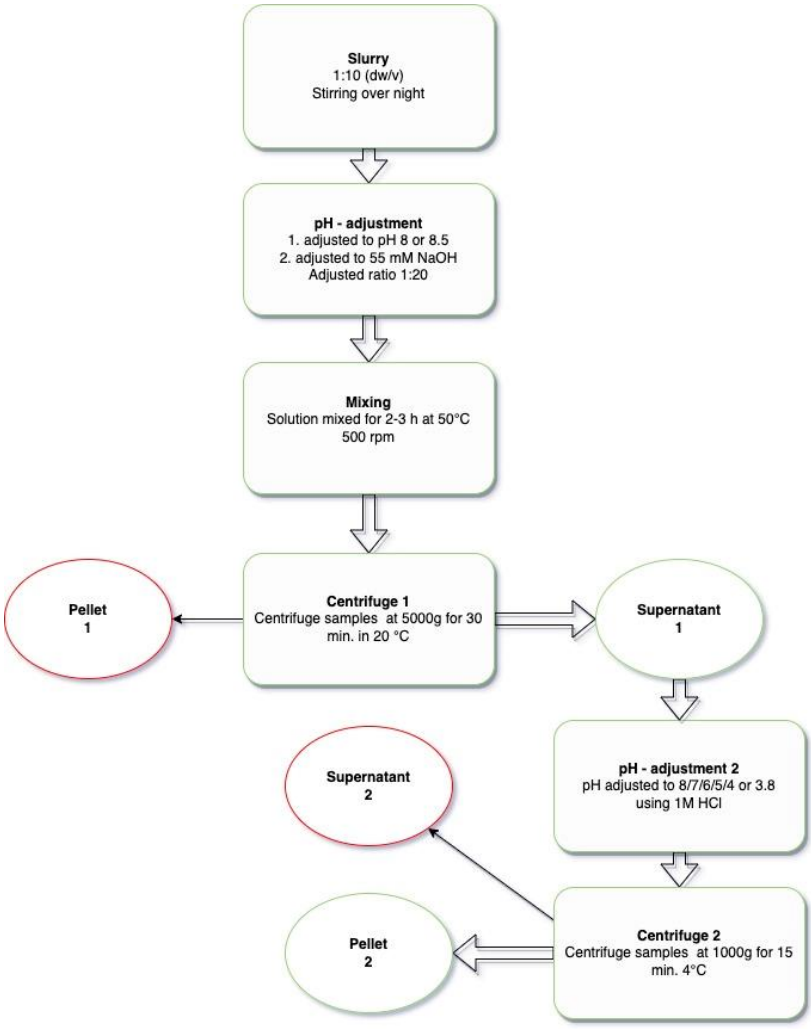


Figure 3. Schematic outline of the extraction process of protein

3.2.1 Alkali-soluble acid precipitate

The extraction processes of the BSG proteins were developed through the experiments. Based on the obtained results, the method was modified for improvement. Varying parameters were pH and temperature. The different stages below describe the variations.

Step 1
The flour was mixed over night with distilled water in a ratio of 1:10, dry weight to volume. Blending was accomplished by a magnetic stirrer, an IKA-COMBIMAG RET or similar, at a speed of approximately 500 rpm.

Step 2
After mixed overnight, the slurry was adjusted to an alkaline pH by 1M NaOH. The adjustment was done with the variations of (a) pH 8, (b) pH 8,5 and (c) 55 mM NaOH, in the slurry. Together with the (c) variation, an extended modification was done introducing L-cysteine (c*). In all cases of a, b, c, and c*, the volume was

adjusted to a ratio of 1:20 (dry weight: volume) by distilled water. After pH-adjustment the slurry was held for 3-4h while mixed by magnetic stirrer at approximately 700 rpm and at temperatures of (d) 50°C, (e) 40°C and (f) room temperature (not higher than 22°C).

Step 3
The slurry was centrifuged at 5000 g for 30 min at 20°C, after mixed for 3-4 hours. Resulting in an alkaline supernatant (S1) and a pellet (P1). The S1 was collected for further experimenting.

Step 4
The supernatant (S1) was treated with 1M HCL to lower the pH, with variants of (g) pH 8, (h) pH 7, (i) pH 6, (j) pH 5, (k) pH 4 and (l) pH 3.8. The acidified supernatant was centrifuged once again at 4°C, 1000g and for 15 min. Both centrifugations were carried out using a Thermo Scientific Sorvall Lynx 4000 or 6000 centrifuge, together with a Fiberlite™ F10-4 x 1000 LEX Fixed Angle Rotor or a Fiberlite™ F9-6 x 1000 LEX Fixed Angle Rotor, respectively A pellet (P2) was collected, and a sample of the supernatant (S2) was saved. Samples from (P1), (P2) and (S2) was frozen and stored in freezer until further treatment at -18°C.

Step 5
The obtained pellet from the last centrifugation (P2), was freezed and thereafter freeze dried for > 48h using a Scanvac Coolsafe at -105°C.

3.2.2 Protein determination

Bradford assay
The freeze-dried protein isolates were further analysed using a Bradford assay. This to determine the protein content in relation and comparison between the different precipitates. Bio-Rad Protein Assay, with the protocol for microtiter plate and together with associated materials were used. A standard curve was prepared by a BSA protein standard in 5 dilutions with a linear range between 0.05 to 0.5 mg/ml.

Protein determination was done in four trials, where the two first differed slightly from the two last sets, in question of preparation. The two first trials were prepared by mixing the precipitate with 20 mM Tromethane hydrochloride (Tris-buffer, Trizma® hydrochloride), the buffer was adjusted to pH 9 but only before added to the precipitate. In the last two experiments the concentration of Tris-buffer was increased to 50 mM. Buffer was added together with NaCl, which was added to a concentration of 100 mM and with the precipitate included, the pH of the blend was adjusted to pH 8.5. Before diluted for the analysis the two last trials were held on a seesaw in room temperature for approximately 2h while dissolving and then centrifuged in a HERAEUS Pico 17 centrifuge (Thermos electron corporation), to remove any unsolved particles.

When the dissolving preparations were done, the samples were diluted in 5-3 steps in a range between 1 mg/ml – 0.05 mg/ml of the protein precipitate. The protein standard and protein samples were loaded in to a 96-well microtiter plate with 10 µl sample in each well. The samples were made in duplicates and as blank

sample, the Tris-buffer solution from the preparation step was used. In each well 200 µl of dye reagent was added. The dye reagent was prepared in a ratio of 1:4, dye reagent to distilled water and filtered through a Whatman #1. Incubation of the samples was carried out for approximately 5-10 minutes but no more than 1h. The plates were read and analysed in a BioTek EON plate reader (BioTek Instruments, Inc) at 595 nm running the Gen 5 software (BioTek Instruments, Inc).

The result from the Bradford assays were used in the estimations of protein concentrations during characterization, as described below.

Kjeldahl analysis

A Kjeldahl analysis was done for two of the precipitates, one from X and one from Y, to get a better understanding of the protein concentration and as a reference for the results from the Bradford assay. The Kjeldahl analysis was performed at another department at the Swedish University of agriculture, *Department of Animal nutrition and management.* The results were obtained directly from them. A protein conversion factor of 6.25 was used, as previously used in Connolly et al. (2013).

3.3 Protein characterization

3.3.1 SDS-Polyacrylamide gel electrophoresis

One run of SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed and included in this project. Protein samples for the run were prepared by letting the precipitates dissolve in Tris-buffer (20 mM Tris-HCL and 100 mM NaCl at pH 9), precipitate concentrations were set to 1, 0.1, 0.01 and 0.001 mg/ml. The protein samples in the Tris-buffer were held on a seesaw for approximately 2h before further treatment. In the next step, the dissolved protein samples were mixed with three parts SDS-buffer to one part protein solution. The SDS-buffer was prepared by mixing 50 µl of mercaptoetanol with 450 µl of 4xSDS. Mixed samples with both Tris-buffer and SDS-buffer were boiled for five (5) minutes at approximately 100°C and then centrifuged at 10 000g. The prepared samples were then loaded on Mini-PROTEAN TGX gels and separated at constant voltage during the electrophoresis.

3.3.2 Gel permeation chromatography

Gel permeation chromatography filtration analysis was carried out on one sample, represented by the Y – flour. The precipitate of the Y flour was mixed with 2mM DTT (dithiothreitol), a bicine buffer of 25 mM and 50mM NaCl. The slurry of buffer and precipitate was adjusted to pH 9, centrifuged to purify the sample and at last filtered through a 0.45 µm PES [polyethersulfone] filter (VWR International). 0.5 ml of the protein solution was injected on a Hiload 16/600 Superdex 200 gel filtration column, (Cytiva Life Sciences) and the protein separation was done at a flow of 0.5 ml/min controlled by an Äkta purifier HPLC system (Cytiva Life Sciences).

3.3.3 Gelation properties

Gelation properties were examined by incubating dissolved protein extracts at 95°C for 30 minutes in a waterbath. The samples were placed in glass tubes with a diameter of 11 mm. The bottom end of the tube was sealed with a rubber lid and the top end was covered with Teflon tape.

Preparation of the precipitates were conducted in different ways to evaluate different circumstances in which gel could be formed. The precipitates were dissolved in (a^G) water, (b^G) 20 mM Tris-buffer adjusted to pH 9 and (c^G) 50 mM Tris-buffer adjusted to pH 9. Adjustment to a pH around 9 was chosen as the protein isolates dissolved around that value rather than a lower pH. The gelation properties were examined in four trials.

The first trial was carried out for X and Y - precipitate and in a concentration of 10%, 20% and 30% in a ratio of weight towards the solvent. In the first trial both water (a^G) and the (b^G) buffer was used, where water was only coupled with the 20% concentration. All samples were done in duplicates for the first trial. The precipitate and solvent were mixed directly in the gel-tubes.

For the second trial, the ratio of precipitate to solvent was set to 10% and only the (c^G) 50 mM Tris-buffer adjusted to pH 9, was used. In this experiment, both precipitates of flour X and Y were tested, and specifically also the precipitate exposed to L-cysteine when extracted. Samples made in duplicates, except the L-cysteine sample.

In the third and fourth trial the (c^G) – 50 mM Tris-buffer, pH 9, buffer was used. Instead of aiming for a certain concentration of the precipitate an estimation was done and the aim was to have 6% of protein. In the last three trials the preparation step was extended: the slurry of buffer and precipitate was adjusted to a pH between 8.39 to 8.72 and were held on a seesaw for around 2h in room temperature prior to the incubation in the water bath. The third trial examined one of the samples: the standard precipitates of the Y flour. The fourth trial included precipitates from both X and Y - flour.

Visual examination was done directly after water bath and when the samples had been allowed to cool down to room temperature. The gel was cut to a hight of 7.5 mm and with a diameter of 8 mm and a one force-examination was performed using a texture analyser (Stable Micro Systems, TA-HDi, Surrey, UK) equipped with a 500 N load cell and a 36 mm cylindrical aluminium probe.

3.3.4 Foaming properties

Foaming properties was examined in two trials, with two samples in each set. The trials were prepared by dissolving the precipitate in (a^f) 50 mM Tris-buffer with 100 mM NaCl or (b^f) only 50 mM Tris-buffer. The first set was prepared by dissolving the precipitate, from both X and Y flour, in a^f and with a protein concentration estimated to 2%. In the next trial X and Y was included again but represented by another extraction trial. b^f was used in the second round and the concentration of protein was estimated to 0.5%. In both trials, pH-adjustments were preformed and aimed for a final pH-value of 9. 1M of NaOH and NaCl was used for the adjustments.

In both cases the volume was 20 ml, and the foaming was executed by using an ULTRA-TURRAX T25 for 5 minutes at 9500 rpm. The samples were held in graduated beakers. Examination of the foam was done at t=0 which was directly after foaming and then at t=5, 15, 30 and 120 min from t=0. The foaming capacity was calculated as the increase of foam from before and after foaming. The stability was evaluated as the decrease in percentage from t=0 until t=120 min.

3.3.5 Protein nanofibrils

When executing the experiment of protein nanofibrils, the precipitate was firstly mixed in water. The slurry was then adjusted to pH 9 with 1M NaOH for the proteins to become soluble and later adjusted to a pH of 2 by using 1M HCl. Two samples were prepared each representing X or Y – flour. The ratio of precipitate to liquid was 1:13, aiming for a protein concentration of 2-3% (w/v) in the slurry and calculated using results from the Bradford assay. The samples were then placed in an oven at 85°C for 18h.

The prepared and heat-treated samples were analyzed by using an AFM microscope (atomic force microscopy). A part of each sample was diluted 10 and 100 times. A droplet of all the different dilutions, original, 10x and 100x, was added to glass slides prepared with plates of silica.

3.3.6 Statistical analysis

The statistical analysis in this study was done using excel and by preforming a two sample T-test, with equal variance and a 95% confidence interval. The test was performed on two groups, identified as the precipitates of the extractions from BSG X and BSG Y. Only the two groups of precipitate-varieties (X and Y) were statistically analysed.

4. Results

4.1 Protein content

In total 16 protein extractions were carried out through this project, divided in to 7 extraction rounds. The extraction rounds are referred to as numbers 1 to 7 and the specific extraction as subheadings to them. This to visualize the historical order of the extractions.

In 14 of the 16 extractions a pellet was obtained able to be analysed for protein content. The results of the protein content from each extraction that resulted in a precipitate are shown in figure 4 and is expressed in percent in relation to the precipitate and the flour. The protein concentrations are all estimated according to obtained results from the Bradford assay, if nothing else is given. The range given for the results is referring to the difference between the duplicates made when doing the Bradford assay. The raw data from the extractions and the calculations is found in table 2 in the appendix.

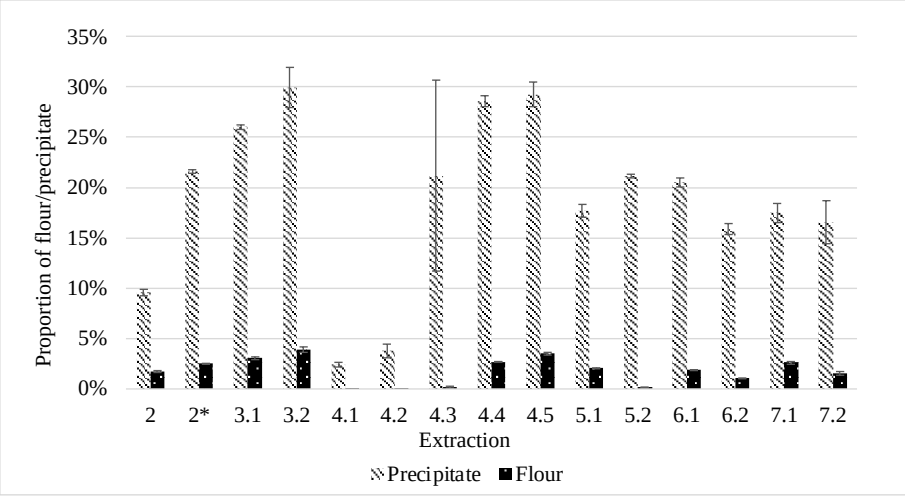


Figure 4. Protein concentrations from the different extractions.

(2) Standard extraction, (2) same as extraction 2 but new Bradford analysis, (3) standard procedure with X and Y, (4) standard but precipitated at pH 8 to 4, (5) standard and addition of L-Cysteine, (6) standard but incubated at 40°C resp. room temperature and (7) standard method both X and Y*

The first extraction (1.1-1.2) was carried out using a pH of 8.5 at the step 2 of both the X and Y flour and did not result in any pellet. Due to the absent pellet and the next extraction (2) was modified according to methods used in Connolly et al., (2013). A higher concentration of NaOH was used to solubilise the proteins in the supernatant, instead of aiming for a pH of 8.5. Non the less, in the extraction two (2) a concentration of 55 mM NaOH was used, and the slurry had a final pH of 12.82.

Extraction two (2) produced a pellet with a dry weight of 11.57 g. The pellet from extraction two (2) showed a protein content of 9.6 ± 0.32% in the precipitate and 1.7 ± 0.06% in the flour (dry weight). In extraction three (3.1-3.2), both flour of BSG dried at 50-60°C (Y, 3.1) and 100-125°C (X, 3.2) was used. The extraction was proceeded with the same method as in extraction two (2) and also called the "standard" method of these experiments. The extraction resulted in a protein content of the precipitate of 26 ± 0.22% (3.1) and 29.9 ± 1.98 % (3.2). The extraction 3.1 showed a concentration of 3.04 ± 0.12% in the flour and 3.2 had a concentration of 3.88 ± 0.26 % in the flour.

When preparing the samples for the Bradford assay, the precipitate from extraction two (2) and three (3.1-3.2) were treated slightly different. Both precipitates were dissolved in 20 mM Tris-buffer and 100 mM NaCl, for the precipitate from extraction two the pH of the buffer-solution was regulated to 8 and in the case of extraction three the pH was adjusted to 9 in the buffer.

Due to the modification of the pH when dissolving the precipitate between the different Bradford assays, the precipitate from extraction two (2) was re-done. The new analysis showed that extraction 2 gave a concentration of 21.6 ± 0.2% in the precipitate and 2.52 ± 0.02% in the flour.

pH when precipitating

Extraction 4 was an experiment to evaluate the pH dependence of protein solubility in which the last pellet was precipitated at 5 different pH-values: 8, 7, 6, 5 and 4. The results from extraction 4 is visualized in figure 5.

The standard method had a pH of 3.8 when precipitating the protein in the last step and the fourth extraction gave an insight in the accuracy of this choice. Extraction 4 showed an increased yield of perception and protein concentration with a lower pH. At pH 8 and 7 the precipitate was very small but large enough to use for Bradford assay. The result showed a very low concentration in both cases, 2.4 ± 0.23% and 3.8 ± 0.67% respectively in the precipitate. At pH 6 the concentration increased to 21.2 ± 9.48% until reaching its highest concentration from pH 4, with 29.2 ± 0.52% protein in the precipitate and 3.4 ± 0.15% in the flour. The decrease of pH used for precipitation appear to increase both the protein concentration in the precipitate but also the total protein obtained which gives a higher protein concentration of the BSG used in each trial (Y in trial four). Judging the results from extraction 4 and the other results from the previous extractions, the value of pH 3.8 seemed to be eligible. Further in the study made by Connolly et al. (2013), they evaluate the addition of reducing agents, L-cysteine, N-acetyl-L-cysteine and B-mercaptoethanol. To develop the extraction of the current study the

addition of reducing agent was of interest. Having L-cysteine available at the lab, this was introduced in a following extraction after the fourth trial.

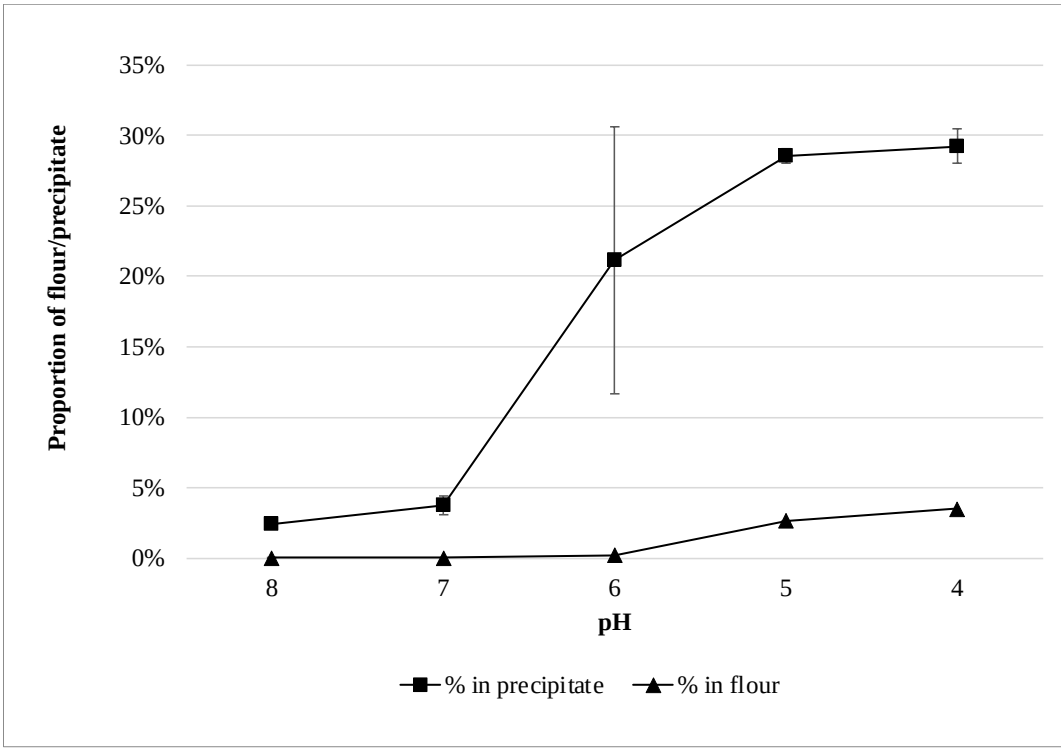


Figure 5. Concentrations in precipitates and flour from extraction 4, precipitation at different pH and including the standard deviations from the Bradford Assay.

Addition of L-cysteine

In the fifth extraction (5.1-5.2) the original method was compared to the addition of L-cysteine. L-cysteine was added as a reducing agent in similarity to what Connolly et al. (2013) did in their study. In Connolly et al. (2013) it was shown that L-cysteine increase the yield of protein, but also that N-acetyl-L-cysteine (NAC) gave an even higher yield. Other than the addition of L-cysteine in 5.2, the two extractions (5.1 and 5.2) were carried out to be similar. Anyhow, the L-cysteine contained HCl and gave the slurry of extraction 5.2, a pH of approximately 7 instead of ≈ 12.89 as in slurry 5.1. Extraction 5.1 resulted in a protein content of 17.7 ±0.63% in the precipitate and extraction 5.2 had a concentration of 21.1 ± 0.2%. This indicated that the addition of L-cysteine gave a higher concentration in the obtained precipitates. Despite the higher concentration in the precipitate the total mass of the precipitate was considerably lower with L-cysteine, 0.5g compared to 7.49 g without the L-cysteine. Due to the different precipitate masses the protein content in relation to the flour differentiated quite a bit. From 5.1 the content showed a value of 2.04 ± 0,07 % protein in the flour and 5.2 resulted in 0.16 ± 0.002 % in the flour. In consideration of the different pH-values in the initial solubilizing-step and the results shown in extraction 1-4. The small precipitate obtained from 5.1 and addition of L-cysteine could be explained by the pH, when comparing to extraction 4.

Temperature

In extraction six (6.1-6.2) and seven (7.1-7.2) the standard method was use. Due to a heating-error in the hotplate magnetic stirrer, the temperatures of the heating step became different. In extraction 6 only flour of the X BSG was used, 6.1 was heated at 40°C for 3h and 6.2 was held at room temperature for the same period. The results showed that 6.1 gave a protein content of 20.5 ± 0.46% in the precipitate and in the flour 1.89 ± 0.04%. Looking at the sample treated at room temperature (6.2) the results gave a content of 15.9 ± 0,56% and 1.04 ± 0.04% in the precipitate and flour respectively.

Extraction 7 was done using both flour from X and Y BSG. 7.1 refers to Y and 7.2 to X. During extraction 7.1, the slurry was exposed to the wanted temperature of 50°C and 7.2 was exposed to 30-50°C due to an error. In the end the extraction 7.1 resulted in 17.5 ± 0.94% and 7.2 at 16.5 ± 2.16% of protein in the precipitate. When calculating the concentration in the respective flour, 7.1 showed a concentration of 2.58 ± 0.14% and 7.2 had a concentration of 1.51 ± 0.2%.

Kjeldahl analysis

As a comparison to the results obtained from the Bradford assay, two samples (A and B) of the precipitates were sent for Kjeldahl analysis. Sample A is represented by extraction 5.1 and sample B by extraction 6.1. Both were extracted according to the standard procedure and representing the X and Y flour respectively. Extraction 6.1 was exposed to 40°C instead of 50°C in the heating step after alkalization.

The Kjeldahl analysis, made in duplicates resulted in a protein concentration of 53.5 % in sample A (X) and 48.7 % in B (Y), in the analysed precipitates. Sample A was extracted to a precipitate of 7.6 g and sample B had a weight of 8.4 g, both had a dry weight of the flour of 65 g. Calculating these values gives a final protein concentration in the X flour of 6.3% and Y flour of 6.2%. This indicates that the results from the Bradford assay at least were not underestimating the concentration.

4.2 Statistics

In this project the X BSG and Y BSG is compared to each other to find out if the amount of extracted protein is statistically different between the groups. In table 1 the results, means and standard deviation is stated. The mean protein content in the precipitate was 21 ± 6% in the X BSG and 20 ± 4% in the Y BSG. The means of the concentration of protein in the flour was for X, 2 ± 1% and for Y 3 ± 0%.

Results from the different extractions were differentiating slightly in method and modified parameters. Most of the variants of extractions was performed as a single trial and have not been made in duplicates or triplicates, able to be compared to each other. This project has two groups that have been analyzed along each other, flour of X BSG and flour of Y BSG. Included in these groups are all the extractions that has been done with the “standard” procedure and the once that only differ in

temperature. Table 1 shows the included values from extraction 2, 3.1, 3.2, 5.1, 6.1, 6.2, 7.1 and 7.2. The only extractions not included is the one treated with L-cysteine and the trial of differentiating pH during acidification.

Table 1. Values from extractions usung X and Y BSG that have been used in the statistical analysis.

Extraction X	Protein conc. % in precipitate	Protein conc. % in flour	Extraction Y	Protein conc. % in precipitate	Protein conc. % in flour
3.2	30%	4%	2*	17%	3%
6.1	20%	2%	3.1	26%	3%
6.2	16%	1%	5.1	18%	2%
7.2	17%	2%	7.1	17%	3%
Mean	21%	2%	Mean	20%	3%
Standard-deviation	6%	1%	Standard-deviation	4%	0%

Not considering temperature as a parameter when comparing the groups, the hypothesis was to find out if there is any statistical difference between the groups. With a double-sided T-test of the two groups and with equal variance, no statistical difference was found as the p-value was > 0.05.

4.3 Characteristics

4.3.1 SDS-Polyacrylamide gel electrophoresis

To visualize the size and distribution of the obtained proteins, an SDS-page was made, using precipitate from extraction 2. The result is showed in figure 6. The ladder used for this SDS-page was in a range of 10 – 250 kDa. The marks from the samples and the ladder were difficult to identify with the obtained picture. Nonetheless, the marks at least indicates that the proteins from extraction 2 are ranging between > 250 kd to <10 kd.

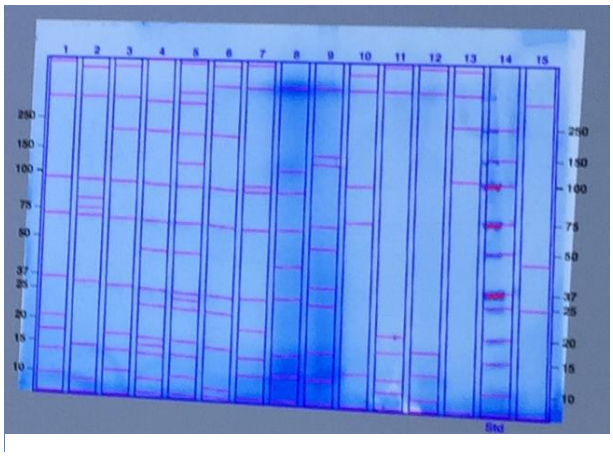


Figure 6. Picture of SDS-PAGE gel where well 8 and 9 was representing dilutions of the proteins in the precipitate from extraction 2.

4.3.2 Gel permeation chromatography

To further analyse the protein distribution in size, a gel chromatography was done. See figure 7 for the obtained results, observe that due to the presence of DTT there was a background absorbance of about 150 mAU. The results showed that there was a large proportion of proteins that passed through the column together and was eluted at the same time. Based on the elution time, it was indicated that the proteins or protein complexes were large, possibly two peaks corresponding to two different proteins or protein complexes. The elution time also indicated that the size of the proteins was at or close to the upper separation limit of the column, which indicated that the proteins were >200 kDa. No other recognisable peaks in the separation part of the elution profile could be identified. The lack of other peaks in the separation indicated that there was not a broad spectrum of protein-size-variations. Close to the end of the elution, there were some peaks which could correspond to small peptides and single aromatic amino acids. When the final particles are eluted, another sample was injected (not related to this study), the conductivity decreases.

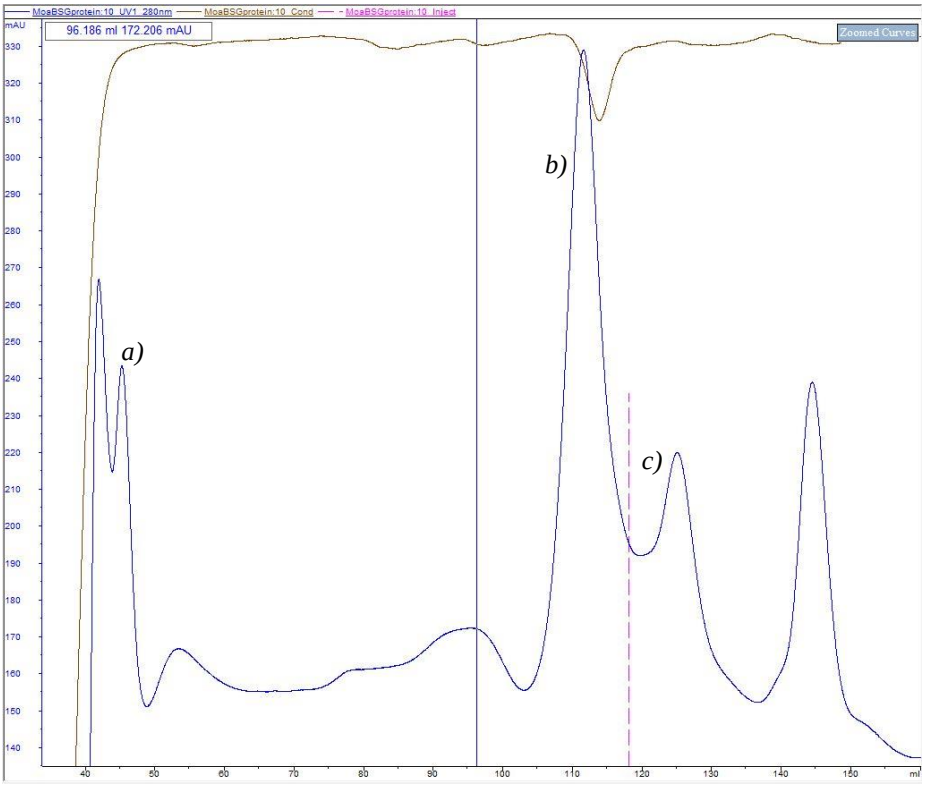


Figure 7. The result of the gel chromatography.

The blue undulating line shows the elution of injected sample of this study. The brown line showed the conductivity. Due to presence of DTT there was a background absorbance of about 150 mAU.

- *a) These peaks indicated proteins at the upper separation limit of the column. Proteins >200kDa.*
- *b) Peaks eluted after 100 ml on the x-axis, indicated on small sized peptides or even single aromatic amino acids.*
- *c) The dashed pink line is showed the injection of another sample, not related to this study.*

4.3.3 Ability to form a gel

Figure 8. Picture of the gel obtained from the gel-round three

In total four trials of gel formation were carried out, using precipitate from extraction three (3.1-3.2), five (5.1-5.2), six (6.1-6.2) and seven (7.1 – 7.2). In the first gel trial, precipitate from extraction three was used. The precipitate was dissolved in ratios of 10, 20 and 30% in Tris-buffer 20mM (pH 9) and water (pH 9). Nether of the samples did form a gel. When using the precipitate from extraction five and six, including the precipitate treated with L-cysteine, a proportion of 10% of precipitate was used. In this trial all the samples were dissolved in 50 mM Tris-buffer and adjusted to pH 9. This trial did not result in any gels either. In the third trial the concentration of precipitate was increased to aim for a protein concentration of 6%. The protein concentration was calculated with the results from the Bradford assay, which means that the percentage is only an estimation. The third trial was carried out using only precipitate from extraction 5.1 and from the Y BSG. The slurry of 50 mM Tris-buffer and precipitate were adjusted to pH 8.39. Round three did result in a gel, see figure 8. In figure 9 a force measurement is showed, which was done on the obtained gel. The analysis is a strength measurement of the gel, possibly useful if studying similar gels in the future.

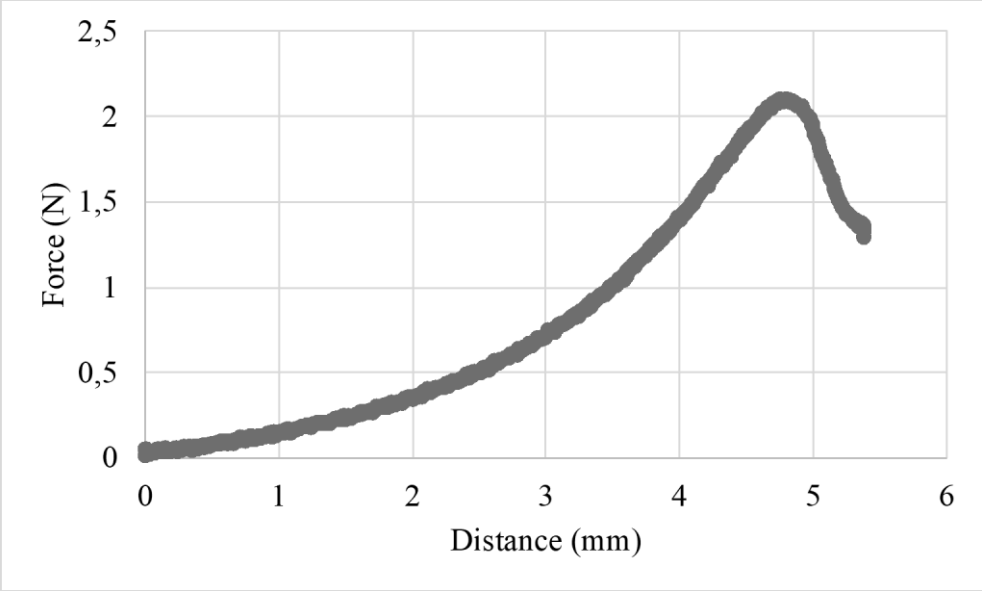


Figure 9. The force measurement of the gel obtained from precipitate 5.1, BSG Y

The last round was carried out using precipitate from extraction seven (7.1-7.2). Extraction seven represented both flour from BSG X and Y. In this round only the sample of extraction 7.1 and BSG Y created a gel, even if both samples were treated the same (50 mM Tris-buffer, pH adjusted to 8.69 and 8.72) and had the same estimated protein concentration of 6%.

In conclusion of the results obtained from the gel ability experiments, two of the samples created gels at a protein concentration of 6% and both represented the BSG Y (50-60°C). The samples of BSG X (100-125°C) did not result in any gel formations.

4.3.4 Foaming

Foaming was tested in two trials, divided on two samples per trial. The first trial (round 1) was done on the precipitate from extraction two (2), BSG Y and on BSG X from the third extraction (3.2). The results are shown in figure 11 (found to the left).

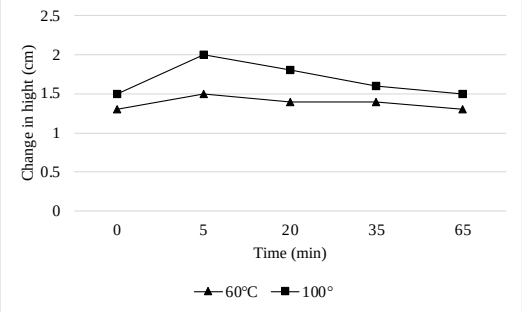


Figure 11. Showing the forming increasement and stability over time, for round 1

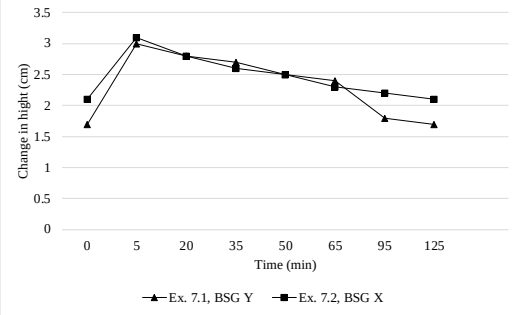


Figure 11. Showing the foaming increasement and stability over time, for round 2

In the next round (round 2), precipitate from extraction seven (7.1-7.2) was used. The results obtained from this round is found in figure 10 (found to the right). Both the trials showed a minimal foam development and with a poor stability. The second trial had a lower protein concentration estimated to 0.5% and an indication of improvement was seen as the foam increased slightly more than in round 1.

4.3.5 Protein nanofibrils

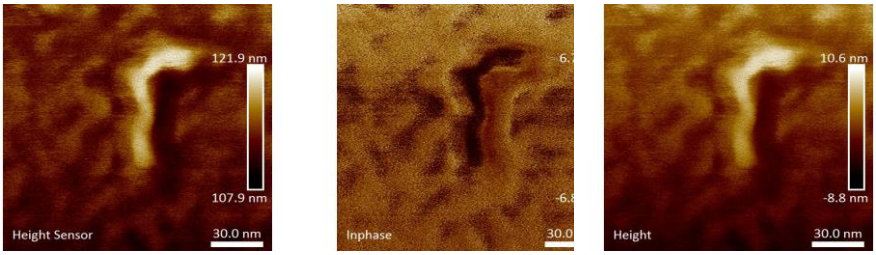


Figure 12. Pictures from the AFN and may visualize a PNF structure All three pictures show the same structure.

The ability to form Protein nanofibrils (PNF) were analyzed by exposing precipitate from extraction seven (7.1 – 7.2) to a rapid acidification and heat treatment. The goal of the treatment was to hydrolyze the proteins in to PNFs. The treatment was not entirely successful and the samples rather coagulated. Figure 12 show what could be a PNF. The picture was taken from the sample of precipitate 7.1 and dilution x100. Even with the obtained picture, no conclusion about the proteins' ability to form PNFs was able to be drawn. The precipitate used in this experiment was probably not pure enough to create the PNFs.

5. Discussion

5.1 Protein content

The aim of this study was to extract protein form brewers' spent grain and to characterise the protein's ability to form foams and gels. The study made by Connolly et al. (2013), referred to in this thesis in the development of methodology, showed that they could extract approximately 23% of the protein from the BSG (dry weight). In their study they used wet BSG as a raw material, which contrasts with this study where dried BSG has been used. This study showed concentrations in the BSG between app. 0 – 4% (dw) when using the results from the Bradford analysis. The variation of the results from the Bradford analysis could be due to the different pH-values in the buffer used when dissolving the proteins, even though the same extraction method was used, as the protein solubility is highly dependent on pH. When looking at the results from the Kjeldahl analysis the concentration is higher compared to results from Bradford but still lower than the concentrations obtained in Connolly et al. (2013). Results from Kjeldahl showed concentrations 6.3% and 6.2%, in the dw BSG flour. Important to consider in Connolly et al. (2013), is that their study also differs from this study in certain parameters within the method. One very important difference was the concentration of NaOH when adjusting the pH of slurry at the step 2. In Connolly et al. (2013), their extractions showed that a concentration of 200 mM gave the highest extraction levels when studying concentrations between 50-200 mM of NaOH. One could argue that 200 mM should be the concentration to aim for, but even Connolly et al. (2013) chose to use 110 mM instead. Looking at other studies it has been shown to be applicable with a concentration of 100 mM as well (Celus et al. 2007). The reason to use lower concentrations is based on minimizing the utilisation of chemicals, in this case NaOH. Given the circumstances of that chemicals should be used with caution, this study tried to show results from a lower concentration then 110 mM and worked with 55mM.

In this study no pretreatment other than the milling was used to increase the possible yield of protein from the BSG, which could have been an interesting factor to investigate. The intention of possibly introducing pretreatments in the future, would be to purify and increase the protein content in the precipitate. Different variations of pretreatment have been reported by (Qin et al. 2018) and Connolly et al. (2013) also examined pre-treatments as hydrochloric acid, acetic acid and hydrogen peroxide. In Connolly et al. (2013), the use of shearing as a pre-treatment showed to increase the yield of protein where they applied 11 000 rpm for 20 and

60 sec. In the study of Qin et al., (2018) they concluded that the use of pre-treatment could increase the yield of protein, but not unproblematically. The use of alkali and diluted acid increased the yield sufficiently in their experiments but it also increased the concentration of carbohydrates and lignin (Qin et al. 2018). In our study the same pattern could be considered as an alkali-soluble acid precipitation was used. The obtained precipitate in this study had a protein concentration between app. 15% and 53.5%, depending on the analytical method (Bradford or Kjeldahl). Anyhow, the results show ability of improvement, considering purification. In a review about arabinoxylans of wheat bran in bread making and treatments that have shown to affect them, they describe a similar extraction method for arabinoxylans as the one used for protein in this study (Pietiäinen et al. 2022). When looking at the results obtained through the standard method and the addition L-Cysteine, two cases of pH differences (55 mM NaOH compared to pH around 7), contrasts in color and protein concentration was observed in this study. Due to the differences in obtained color and protein concentrations, it may be discussed that a higher pH and concentration of NaOH increases the extraction of arabinoxylans and maybe other fibers as well. The conclusions in Qin et al., (2018) and the method outlined in Pietiäinen et al. (2022), could be a part of the reason our extraction did not result in a more pure protein precipitates. Further, a modification of the extraction process would probably benefit of taking this into consideration.

In the elongation, the question could be if a purified protein isolate is needed and especially as an ingredient in food. Well, both a purified sample and a blended protein sample would need studies of applicability. For studying, an isolated protein sample usually makes the analyses easier and on the other hand maybe a non-purified sample could be of more interest in a food product. Important to investigate equally for the different cases would be the possible presence of antinutrients, which may have a higher concentration in a non-purified sample (Farag et al. 2022).

5.2 Characterization

In the current study, characterization of the proteins was analyzed using SDS-page, gel chromatography, foaming and gelling analysis as well as looking into the ability to form PNFs. When comparing the results from the SDS-page and gel chromatography with Connolly et al. (2013), both similarities and differences are found. On the SDS-page, Connolly et al. (2013) observed that their protein seemed to have a range between 71 – 240 kDa. In the case of this stud, the range was not so clear but visible lines were observed between 10 – 250 kDa. The observation in this study seems to be a bit on the outer range of what could be expected when comparing to the study by Connolly et al. (2013). The inequalities may be caused by the different methods, materials and which may cause different characteristics of the precipitates and bonding between the proteins when analysing. In Connolly et al. (2013), referring to Celus et al. (2006) , they describe that the B and C variants of the hordeins are of a size within the range of 42 – 71 kDa. The results obtained from Connolly et al. (2013) and in this study, indicates that B and C hordeins could at least be present to some extent, because of the results from the different size exclusions. The presence of B and C hordeins is not unpredictable as they contribute to the majority of the protein within the barley (Shewry et al. 1983). Important to

remember is that the knowledge about the BSG in the study made by Connolly et al. (2013) is limited and it is therefore difficult to clarify the differences between BSG in this study and theirs. Maybe the BSG have been treated more extensively during the brewing process with higher temperatures or longer time in one of the studies. Theoretically, the time of malting should increase the availability of starch in the mashing and maybe the proteins follow this pattern as they adhere to the carbohydrates, especially with higher roasting temperatures.

The gel chromatography in this study gave a profile as seen in figure 7, which compared to the profile obtained in Connolly et al. (2013) looks similar at first sight. Connolly et al. (2013) also referred to the similarity to the results obtained from Celus et al. (2007). Both studies could identify the profile coupled with molecular weight and Connolly et al. (2013) found that they had a more than half of the proteins with a molecular weight of >10 kDa. In this study no specific molecular weight was applicable to the peaks, due to the choice of method and combination of characterisations. On the other hand, the column used in this study had a capacity where the proteins should be separated from 200 kDa or below. The peak from this study eluted in the very beginning which indicates that the present proteins were of a size ≥200 kDa. Furthermore, this study used 280 nm and Connolly et al. (2013) as well as Celus et al. (2007), used 214 nm. In Connolly et al. (2013) they do not mention the specific column used for their separation, in Celus et al. (2006) it seems like they used a column able to separate proteins below 100 kDa. Studying the column and the obtained profile it may be discussed that they could have proteins that are larger than 100 kDa. The results from Connolly et al. (2013) and Celus et al. (2006), were referring to methods different from the one in this study and the descriptions were slightly different, but the visual results seed to have similarities.

When studying the ability to form gel and foam, the range of reference material was not huge. The possibility to compare the obtained results in this study, with other previous studies were limited. The foaming ability is an important characteristic when evaluating proteins from a food application perspective (Zayas 1997). In the present study the results showed that the proteins in the trials did have the ability to form foams, but they do not seem to be considerably stable. At times, the bubbles formed when foaming is studied, but this has not been done in this project due to time limits and the stability of the foam. When studying the gel formation, it can be concluded in similarity to the foaming, that gels are able to form with the used precipitate in this study. On the other hand, applicable for both the gel and foam, is the impurified samples and they create an uncertainty around the involvement of the other ingoing parts. Assuming that, the Kjeldahl analysis indicates on the right concentration of protein concentration, then the method would generate a protein concentration of app. 50%. Even if the protein concentration was as high as 50% in the precipitates, around 50% is something else and that potentially could both disrupt and encourage the gel and foaming ability. The results from the gel experiments showed that gels were able to form, but in this current study only from the BSG Y, e.g., dried at 50-60°C. The other BSG variant (X), dried at 100-125°C did not show any signs of gelation. In these cases, the BSG has overall been treated the same despite the drying temperature and the extraction has also been similar. The proteins of this study have been pre-treated (dried) in different temperatures depending on the X or Y variant. The interesting part of this, is that 50/60°C and 100/125°C could influence the proteins characteristics. This is both

because the difference in temperature affects the hydration but also the fact that denaturation of protein is dependent on exposure of degree of temperature.

The PNF possibly found in this study was not clear enough to state that the BSG-protein extractions could form PNFs. On the other hand, figure 12 visualized a possible PNF and it can be argued that this will be interesting enough to further study this subject in another study. PNF is an interesting area of use in the food industry as it can also enhance other characteristics. For example the study Akkermans et al. (2008), they showed that whey protein isolates with added PNF did increase viscosity and strength of gel. Using PNF was not only resulting in expected improvement of gelation strength but also shear thickening and phase separation (Akkermans et al., 2008). Anyhow, PNF could be an interesting area of development in the future and if a more purified sample of protein was obtained the creation of PNFs would probably be simplified. In this trial a precipitate of both protein and other particles was used which could have interrupted with the experiments.

5.3 Future research

This study was developed using an approach of exploration, which was useful in this type of subject where earlier studies were limited. Using a broad path when experimenting gave the opportunity to explore many parameters. But as the availability of time was limited, likewise was the ability to explore each parameter on a deeper level. In the stud by Connolly et al. (2013), many of the explored extraction parameters in this study have already been tested and notwithstanding of this the results were quite different. Indicating that more studies need to be performed with different raw materials, to understand the possibility of using protein from BSG.

One important aspect is mentioned in the section about extracting protein and the “side-extraction” of other fractions as carbohydrates and lignin. In this study only the proteins were analysed and included with interest, but further the other ingoing and/or outgoing parts would be a positive aspect to involve. As the protein content in the precipitate and the different BSG was showed to be considerably low in the current stud, it could be argued that protein extraction may not be the target when evaluating BSG as a food source. Maybe further studies should concentrate on the development of extraction methods for carbohydrates and for example arabinoxylans. When the extraction of carbohydrates is optimized, it could be assumed that a side-stream of proteins may occur and then it may be of interest to develop areas of use.

6. Conclusion

In summary of this project, proteins were possible to extract from both studied BSG varieties (X and Y). The concentration of protein in the obtained precipitates did not show to be of the most optimized levels for further analyses. None of the X or Y group showed to have any significantly larger or smaller extraction ratio of proteins. The characteristics of these proteins, judging of the ability to form gel and foam are questionable as the precipitate did contain a considerable proportion of other particles. Nonetheless, the obtained precipitates in this study did show characteristics of being large in size and/or aggregated into larger units. Further, the precipitates did also show ability to form foams, even if the formation and stability was not of any higher quality. The ability to form gels were showed for precipitates of BSG Y but not BSG X.

In the future the extraction would need to be optimized if isolated proteins are of further interest. The isolation is also of importance if the characteristics should be able to analyse as a reaction to the proteins, not the mixture of proteins and other ingoing parts.

References

Akkermans, C., Van der Goot, A.J., Venema, P., Van der Linden, E. & Boom, R.M. (2008). Properties of protein fibrils in whey protein isolate solutions: Microstructure, flow behaviour and gelation. *International Dairy Journal*, 18 (10), 1034–1042. https://doi.org/10.1016/j.idairyj.2008.05.006

Arendt, E.K. & Zannini, E. (2013). *Cereal Grains for the Food and Beverage Industries*. Cambridge, UNITED KINGDOM: Elsevier Science & Technology. http://ebookcentral.proquest.com/lib/slub-ebooks/detail.action?docID=1575058 [2022-04-25]

Barth, R. (2013). *The Chemistry of Beer: The Science in the Suds*. Somerset, UNITED STATES: John Wiley & Sons, Incorporated. http://ebookcentral.proquest.com/lib/slub-ebooks/detail.action?docID=1368917 [2022-01-25]

Britannica, T. (2020). Barley. Encyclopedia Britannica. Editors of Encyclopaedia. https://www.britannica.com/plant/barley-cereal

Bryggerirapporten 2020 (2020). Sveriges Bryggerier.

Celus, I., Brijs, K. & Delcour, J.A. (2007). Enzymatic Hydrolysis of Brewers' Spent Grain Proteins and Technofunctional Properties of the Resulting Hydrolysates. *Journal of Agricultural and Food Chemistry*, 55 (21), 8703–8710. https://doi.org/10.1021/jf071793c

Connolly, A., Piggott, C.O. & FitzGerald, R.J. (2013). Characterisation of protein-rich isolates and antioxidative phenolic extracts from pale and black brewers' spent grain. *International Journal of Food Science & Technology*, 48 (8), 1670–1681. https://doi.org/10.1111/ijfs.12137

Czubaszek, A., Wojciechowicz-Budzisz, A., Spychaj, R. & Kawa-Rygielska, J. (2021). Baking properties of flour and nutritional value of rye bread with brewer's spent grain. *LWT*, 150, 111955. https://doi.org/10.1016/j.lwt.2021.111955

Damodaran, S., Parkin, K.L. & Fennema, O.R. (2017). *Fennema's food chemistry*. Fifth edition. Boca Raton: CRC Press.

Delcour, J.A. & Hoseney, R.C. (2010). *Principles of cereal science and technology*. 3rd ed. Minnesota U.S.A: St. Paul, Minn. : AACC International.

Dryckes bransch rapporten 2021 (2021). LRF Lokal mat och dryck, Föreningen Svenskt vin & Sveriges Producenter Av Alkoholdrycker.

Farag, M.A., Xiao, J. & Abdallah, H.M. (2022). Nutritional value of barley cereal and better opportunities for its processing as a value-added food: a comprehensive review. *Critical Reviews in Food Science and Nutrition*, 62 (4), 1092–1104. https://doi.org/10.1080/10408398.2020.1835817

Fox, G.P. (2009). Chemical Composition in Barley Grains and Malt Quality. In: Zhang, G. & Li, C. (eds.) *Genetics and Improvement of Barley Malt Quality*. Berlin, Heidelberg: Springer Berlin Heidelberg, 63–98. https://doi.org/10.1007/978-3-642-01279-2_3

Jonassen, I., Ingversen, J. & Brandt, A. (1981). Synthesis of SP II albumin, β-amylase and chymotrypsin inhibitor CI-1 on polysomes from the endoplasmic reticulum of barley endosperm. *Carlsberg Research Communications*, 46 (3), 175. https://doi.org/10.1007/BF02910466

Kirkman, M.A., Shewry, P.R. & Miflin, B.J. (1982). The effect of nitrogen nutrition on the lysine content and protein-composition of barley-seeds. *Journal of the Science of Food and Agriculture - jsfa*, 33 (2), 115–127. https://doi.org/10.1002/jsfa.2740330203

Lásztity, R. (1996). *The chemistry of cereal proteins*. 2. ed. Boca Raton, Fla. ; CRC Press.

Mussatto, S.I. (2014). Brewer's spent grain: a valuable feedstock for industrial applications: Brewer's spent grain and its potential applications. *Journal of the Science of Food and Agriculture*, 94 (7), 1264–1275. https://doi.org/10.1002/jsfa.6486

Mussatto, S.I., Dragone, G. & Roberto, I.C. (2006). Brewers' spent grain: generation, characteristics and potential applications. *Journal of Cereal Science*, 43 (1), 1–14. https://doi.org/10.1016/j.jcs.2005.06.001

Nazir, A., Asghar, A. & Aslam Maan, A. (2017). Chapter 13 - Food Gels: Gelling Process and New Applications. In: Ahmed, J., Ptaszek, P., & Basu, S. (eds.) *Advances in Food Rheology and Its Applications*. Woodhead Publishing, 335–353. https://doi.org/10.1016/B978-0-08-100431-9.00013-9

Neylon, E., Arendt, E.K., Zannini, E. & Sahin, A.W. (2021). Fundamental study of the application of brewers spent grain and fermented brewers spent grain on the quality of pasta. *Food Structure*, 30, 100225. https://doi.org/10.1016/j.foostr.2021.100225

Olsson, Y. (2021). *Jordbruksmarkens användning 2021. Slutlig statistik*. (JO0104). Jordbruksverket. https://jordbruksverket.se/om-jordbruksverket/jordbruksverkets-officiella-statistik/jordbruksverkets-statistikrapporter/statistik/2021-10-19-jordbruksmarkens-anvandning-2021.-slutlig-statistik

Pietiäinen, S., Moldin, A., Ström, A., Malmberg, C. & Langton, M. (2022). Effect of physicochemical properties, pre-processing, and extraction on the functionality of wheat bran arabinoxylans in breadmaking – A review. *Food Chemistry*, 383, 132584. https://doi.org/10.1016/j.foodchem.2022.132584

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

Rosentrater, K.A. & Evers, A.D. (2018). Chapter 3 - Botanical aspects. In: Rosentrater, K.A. & Evers, A.D. (eds.) *Kent's Technology of Cereals (Fifth Edition)*. Woodhead Publishing, 205–266. https://doi.org/10.1016/B978-0-08-100529-3.00003-7

Shewry, P.R., Franklin, J., Parmar, S., Smith, S.J. & Miflin, B.J. (1983). The effects of sulphur starvation on the amino acid and protein compositions of barley grain. *Journal of Cereal Science*, 1 (1), 21–31. https://doi.org/10.1016/S0733-5210(83)80005-8

Shewry, P.R., Parmar, S. & Field, J.M. (1988). Two-dimensional electrophoresis of cereal prolamins: Applications to biochemical and genetic analyses. *ELECTROPHORESIS*, 9 (11), 727–737. https://doi.org/10.1002/elps.1150091108

Sipe, J.D., Benson, M.D., Buxbaum, J.N., Ikeda, S.-I., Merlini, G., Saraiva, M.J.M. & Westermark, P. (2016). Amyloid fibril proteins and amyloidosis: chemical identification and clinical classification International Society of Amyloidosis 2016 Nomenclature Guidelines. *Amyloid: The International Journal of Experimental and Clinical Investigation: The Official Journal of the International Society of Amyloidosis*, 23 (4), 209–213. https://doi.org/10.1080/13506129.2016.1257986

Suchkov, V.V., Popello, I.A., Grinberg, V.Ya. & Tolstoguzov, V.B. (1990). Isolation and purification of 7S and 11S globulins from broad beans and

peas. *Journal of Agricultural and Food Chemistry*, 38 (1), 92–95. https://doi.org/10.1021/jf00091a017
UNDP (n.d.). Mål 12: Hållbar konsumtion och produktion. *Globala målen*. https://www.globalamalen.se/om-globala-malen/mal-12-hallbar-konsumtion-och-produktion/ [2022-06-04]
Wang, C., Tian, Z., Chen, L., Temelli, F., Liu, H. & Wang, Y. (2010). Functionality of Barley Proteins Extracted and Fractionated by Alkaline and Alcohol Methods. *Cereal Chemistry*, 87 (6), 597–606. https://doi.org/10.1094/CCHEM-06-10-0097
Waters, D.M., Jacob, F., Titze, J., Arendt, E.K. & Zannini, E. (2012). Fibre, protein and mineral fortification of wheat bread through milled and fermented brewer's spent grain enrichment. *European Food Research and Technology*, 235 (5), 767–778. https://doi.org/10.1007/s00217-012-1805-9
Zayas, J.F. (1997). Foaming Properties of Proteins. In: Zayas, J.F. (ed.) *Functionality of Proteins in Food*. Berlin, Heidelberg: Springer, 260–309. https://doi.org/10.1007/978-3-642-59116-7_6
Zhao, J., Tian, Z. & Chen, L. (2011). Effects of deamidation on aggregation and emulsifying properties of barley glutelin. *Food Chemistry*, 128 (4), 1029–1036. https://doi.org/10.1016/j.foodchem.2011.04.009

Appendix 1 – Raw data

Results and calculations from the specific protein extraction and absorbance measurements:

Extraction	Flour/ method	Sample ID Average and abs. Read 1 or 2	Dilution	Net abs.	conc. (ug/ml)	volume analyed (ml)	mass in analyze (ug)	Volume diluted 1 (ml)	con. Diluted 1 (ug/ml)	volume in dilution 1 (ml)	mass in dilution 1 (ug)	volume in dilution 2 (ml)	conc. dilution 2 (ug/ml)	volume of dissolved protein (ml)	mass of protein in dissolved precipitation (g)	Weight of dissolved precipitate	conc. of protein (ug/g)	Weight of total precipitate (g)	estimated protein precipitation (g)	Amount of protein in precipitate (%)	Weight of flour used (g)	Amount of protein in flour (%)
2	60°C/ standard extraction	Average abs. 1	1mg/ml	1.097	960.5454545									50	0.3009							
			1mg/ml	1.062	928.7272727	50	46436.3636							50	46436.3636	0.3009	92856.0132	11.5742	1.07473407	9.3%	65	1.7%
		abs. 2	1mg/ml	1.132	992.3636364	50	49618.1818							50	49618.1818	0.3009	99218.5943	11.5742	1.14837481	9.9%	65	1.8%
2*	60°C/ same as 2 but new absorbance trial	Average abs. 1	1 mg/ml	0.464	271.5	1	271.5	0.1	2715	0.8	2172	1	2172	10	21720	0.1056	215990.453	7.5934	1.64010191	21.6%	65	2.5%
		abs. 1	1 mg/ml	0.46	269	1	269	0.1	2690	0.8	2152	1	2152	10	21520	0.1056	214001.591	7.5934	1.62489968	21.4%	65	2.5%
		abs. 2	1 mg/ml	0.468	274	1	274	0.1	2740	0.8	2192	1	2192	10	21920	0.1056	217979.316	7.5934	1.65520414	21.8%	65	2.5%
3.1	60°C/ standard extraction	Average abs. 1	1mg/ml	0.529333333	280.3958333			50	13019.7917					50	13019.7917	0.0501	260343.765	7.5934	1.97889434	26.0%	65	3.0%
			1mg/ml	0.531	276.0588235			50	13802.9412					50	13802.9412	0.0501	276003.623	7.5934	2.09598591	27.6%	65	3.2%
		abs. 2	1mg/ml	0.551	287.8235294			50	14391.1765					50	14391.1765	0.0501	287765.976	7.5934	2.18512216	28.8%	65	3.4%
3.2	100°C/ Standard	Average abs. 1	1mg/ml	0.3975	303	50	15150							50	15150	0.05063	299229.706	8.439	2.52519949	29.9%	65	3.9%
			1mg/ml	0.579	304.2941176	50	15214.7059							50	15214.7059	0.05063	300507.72	8.439	2.53398465	30.1%	65	3.9%
		abs. 2	1mg/ml	0.647	344.2941176	50	17214.7059							50	17214.7059	0.05063	340069.992	8.439	2.86934432	34.0%	65	4.4%
4.1	60°C/ precipitated at pH 8	Average abs. 1	1mg/ml	0.103	24.29411765	50	1214.70588							50	1214.70588	0.05015	24221.4533	0.06373	0.00154363	2.4%	8	0.0%
			1mg/ml	0.099	21.94117647	50	1097.05882							50	1097.05882	0.05015	21875.2498	0.06373	0.00139413	2.2%	8	0.0%
		abs. 2	1mg/ml	0.107	26.64705882	50	1332.35294							50	1332.35294	0.05015	26567.3568	0.06373	0.00169314	2.7%	8	0.0%
4.2	60°C/ pre. at pH 7	Average abs. 1	1mg/ml	0.1265	38.11764706	50	1905.88235							50	1905.88235	0.05067	37613.6245	0.05736	0.00215752	3.8%	8	0.0%
			1mg/ml	0.115	31.35294118	50	1567.64706							50	1567.64706	0.05067	30938.3671	0.05736	0.00177462	3.1%	8	0.0%
		abs. 2	1mg/ml	0.138	44.88235294	50	2244.11765							50	2244.11765	0.05067	44288.8819	0.05736	0.00254941	4.4%	8	0.0%
4.3	60°C/ pre. at pH 6	Average abs. 1	1mg/ml	0.4245	213.4117647	50	10670.5882							50	10670.5882	0.05042	211634.039	0.07762	0.01642703	21.2%	8	0.2%
			1mg/ml	0.262	117.8235294	50	5891.17647							50	5891.17647	0.05042	116842.056	0.07762	0.00906928	11.7%	8	0.1%
		abs. 2	1mg/ml	0.587	309	50	15450							50	15450	0.05042	306426.021	0.07762	0.02379479	30.6%	8	0.3%
4.4	60°C/ pre. at pH 5	Average abs. 1	1mg/ml	0.553	289	50	14450							50	14450	0.05057	285742.535	0.74133	0.2118295	28.6%	8	2.6%
			1mg/ml	0.544	283.7058824	50	14185.2941							50	14185.2941	0.05057	280508.09	0.74133	0.20794906	28.1%	8	2.6%
		abs. 2	1mg/ml	0.562	294.2941176	50	14714.7059							50	14714.7059	0.05057	290976.98	0.74133	0.21570996	29.1%	8	2.7%
4.5	60°C/ pre. at pH 4	Average abs. 1	1mg/ml	0.565	296.0588235	50	14802.9412							50	14802.9412	0.05063	292374.9	0.95853	0.28025011	29.2%	8	3.5%
			1mg/ml	0.544	283.7058824	50	14185.2941							50	14185.2941	0.05063	280175.669	0.95853	0.26855678	28.0%	8	3.4%
		abs. 2	1mg/ml	0.586	308.4117647	50	15420.5882							50	15420.5882	0.05063	304574.131	0.95853	0.29194344	30.5%	8	3.6%
5.1	60°C/standa rd	Average abs. 1	1 mg/ml	0.3405	190.0714286	1	190.07429	0.1	1900.71429	1.4	2661	1.5	1774	10	17740	0.1002	177045.908	7.49	1.32687385	17.7%	65	2.0%
		abs. 1	1 mg/ml	0.3065	196.8571429	1	196.85714	0.1	1968.57143	1.4	2756	1.5	1837.33333	10	18373.3333	0.1002	18336616	7.49	1.37341583	18.3%	65	2.1%
		abs. 2	1 mg/ml	0.2875	183.2857143	1	183.28571	0.1	1832.85714	1.4	2566	1.5	1710.66667	10	17106.6667	0.1002	170725.216	7.49	1.27873187	17.1%	65	2.0%
5.2	60°C/with L-cysteine	Average abs. 1	1 mg/ml	0.3485	226.8571429	1	226.85714	0.1	2268.57143	1.4	3176	1.5	2117.33333	10	21173.3333	0.1002	211310.712	0.5026	0.10620476	21.1%	65	0.2%
		abs. 1		0.3515	229	1	229	0.1	2290	1.4	3206	1.5	2137.33333	10	21373.3333	0.1002	213306.72	0.5026	0.10720796	21.3%	65	0.2%
		abs. 2		0.3455	224.7142857	1	224.74286	0.1	2247.14286	1.4	3146	1.5	2097.33333	10	20973.3333	0.1002	209314.704	0.5026	0.10520157	20.9%	65	0.2%
6.1	100°C/water bath at 40°C	Average abs. 1	1 mg/ml	0.3405	221.1428571	1	221.14286	0.1	2211.42857	1.4	3096	1.5	20640	10	20640	0.1007	204965.243	5.9907	1.22788528	20.5%	65	1.89%
		abs. 1	1 mg/ml	0.3475	226.1428571	1	226.14286	0.1	2261.42857	1.4	3166	1.5	2110.66667	10	21106.6667	0.1007	209599.47	5.9907	1.25564755	21.0%	65	1.9%
		abs. 2	1 mg/ml	0.3335	216.1428571	1	216.14286	0.1	2161.42857	1.4	3026	1.5	2017.33333	10	20173.3333	0.1007	200331.016	5.9907	1.20012302	20.0%	65	1.8%
6.2	100°C/Wate rbath at room T.	Average abs. 1	1 mg/ml	0.27	170.7857143	1	170.78571	0.1	1707.85714	1.4	2391	1.5	1594	10	15940	0.1005	158606.965	4.2507	0.67419063	15.9%	65	1.0%
		abs. 1		0.2615	164.7142857	1	164.71429	0.1	1647.14286	1.4	2306	1.5	1537.33333	10	15373.3333	0.1005	152968.491	4.2507	0.65022316	15.3%	65	1.0%
		abs. 2		0.2705	176.8571429	1	176.85714	0.1	1768.57143	1.4	2476	1.5	1650.66667	10	16506.6667	0.1005	164245.439	4.2507	0.69815809	16.4%	65	1.1%
7.1	60°C/ standard extraction	Average abs. 1	1 mg/ml	0.382	220.25	1	220.25	0.1	2202.5	0.8	1762	1	1762	10	17620	0.1079	174818.93	9.399505	1.6781752	17.5%	65	2.6%
		abs. 1	1 mg/ml	0.363	208.375	1	208.375	0.1	2083.75	0.8	1667	1	1667	10	16670	0.1079	165393.392	9.399505	1.58765947	16.5%	65	2.4%
		abs. 2	1 mg/ml	0.401	232.125	1	232.125	0.1	2321.25	0.8	1857	1	1857	10	18570	0.1079	184244.469	9.399505	1.76865577	18.4%	65	2.7%
7.2	100°C/ standard	Average abs. 1	1 mg/ml	0.3625	208.0625	1	208.0625	0.1	2080.625	0.8	1664.5	1	1664.5	10	16645	0.1061	165440.811	5.95108	0.98453515	16.5%	65	1.5%
		abs. 1		0.319	180.875	1	180.875	0.1	1808.75	0.8	1447	1	1447	10	14470	0.1061	143822.682	5.95108	0.85390028	14.4%	65	1.3%
		abs. 2		0.406	235.25	1	235.25	0.1	2352.5	0.8	1882	1	1882	10	18820	0.1061	187058394	5.95108	1.11320272	18.7%	65	1.7%

Table 2. Raw data on the protein extraction and calculations from the Bradford Assay.

Is Brewers' spent grain, the next popular source of plant protein?

Moa Rönnhagen 2022-05-22

Did you know that huge amounts of waste are produced every year from the food industry? Among the food waste and loss, we find Brewers' spent grain, a residual product from beer production. Brewers' spent grain could potentially be a resource of protein, fiber, and other important nutrients.

Background:
Brewers' spent grain is occasionally used as animal feed and as plant nutrition, but more uncommon as food for humans. What if we could transform the fibrous raw material and turn it into something great? Because it is a fibrous material it could be more difficult to incorporate it into new products both practically and sensorily. Therefore, the possibility to extract products as protein is of interest. Protein extraction has been done before but not with the provided brewers' spent grain of this study, and even fewer trials of characterization has been carried out. The thought was to find out if protein extraction is a potential candidate as a future protein resource when developing new food products.

About the study:
This project was a master thesis, made in collaboration with Swefood Upcycling AB providing brewers' spent grain from the brewery, Värmdö Bryggeri. The aim of the thesis was to extract protein from two variants of brewers' spent grain and to investigate their ability to form gels and foams, as well as protein nanofibrils. The extraction was preformed using an alkali-soluble and acid-perception.

Results:
The protein extractions were both successful and questionable. The obtained precipitates did contain protein, but the amount protein and purity of the precipitate made it difficult to evaluate further characterization. Even if conclusions could not be drawn with certainty for the proteins, the experiments of characterization showed that the precipitates did have ability to form gels and foams.

Protein extractions can be performed in many ways and more studies should continue to try for more purification of the precipitate. Even though the protein may not be the target for Brewers' spent grain, maybe it will be a useful side stream of another extraction as valuable fibers.

Acknowledgements

This study would not have been able to carry out if not Niclas Tunebro at Swefood Upcykling AB had provided the project with time and brewers' spent grain from Värmdö bryggeri. For this participation I would like to send my greatest thanks to both Niclas and Värmdö bryggeri.

Further, my supervisor Henrik has been very supportive through the process and have given me help on the way, when needed. A big thank you to Henrik, for the commitment in my work. In extension to Henrik, I would like to thank the whole team at Food structure and properties, including doctoral students, scientists, and master students. All of you have participated in the process with support, coffee, and knowledge.

Without every one of the participants in this project the thesis would not have been the same, thanks to all of you who I have been in contact and worked with.