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Swedish University of Agricultural Sciences

Department of Molecular Sciences

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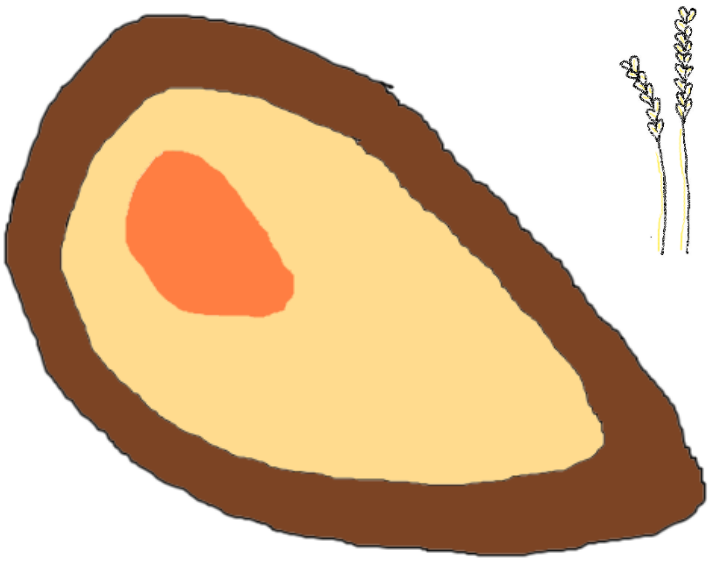
Optimering av stärkelseextraktion för fraktionering av vetekli.

Louise Nybogård



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Abstract

With continuous growth of the human population and limited resources for food production, there is a need to optimize the utilization of already produced food. Wheat bran is the outer layer of the wheat kernel and a side stream product from flour milling, containing multiple potential functional ingredients. A fractionation process for wheat bran with mild and non-destructive extraction methods is needed to keep all compounds intact. The initial process step is generally to de-starch the wheat bran through one swell part with a 1:10 (bran:water) ratio and several washing steps with a 1:6 (bran:water) ratio.

The aim of this project was to decrease the water usage of the de-starching in wheat fractionation, as well as investigate if and to what extent that any functional ingredients are lost into the water used, called slurry, during the process. De-starching was made with three different water ratios (1:6, 1:8, 1:10; bran:water) for the swelling step and three different water ratios (1:4, 1:5, 1:6; bran:water) for five washing steps.

For all bran:water treatments the starch content decreased from 9,00 % to below 1,00 % in the bran after three washing steps. The amount water needed have been narrowed down to 1:6-1:8 (bran:water) for swell water ratio and to 1:5 or below (bran:water) for the wash water ratio, without any impact on the process efficiency. The total water use was thereby decreased with at least 20 %. Small concentrations of arabinoxylans (< 0,4 mg/g slurry) and proteins (<0,3 mg/g slurry) were found in the water extractable part of the slurries. At least 40 % of the water unextractable part was non-starch and have potential to be valuable chemical compounds. Future studies need to focus on the water unextractable part of the slurries from the de-starching process of wheat bran. This project could bring useful information for the industry when planning to de-starch wheat bran and extract its nutrients, leading to a decrease of the environmental impact and targeting the most economically efficient nutrients to extract. Thus, being a part of optimizing the utilization of already produced food.

Keywords: De-starching process, fractionation, functional ingredients, wheat bran

Sammanfattning

Med en ständigt ökande befolkning och begränsade resurser för livsmedelsproduktion så finns det ett behov av att optimera utnyttjandet av redan producerad mat. Vetekli är det yttre lagret av vetekärnan, en biprodukt från malning av mjöl, som innehåller flera potentiella funktionella ingredienser. En fraktioneringsprocess med milda och icke-destruktiva extraktionsmetoder behövs för att hålla alla komponenter intakta. Det första steget är generellt att ta bort stärkelse med hjälp av ett sväll-steg där vattenförhållandet är 1:10 (kli:vatten) och flera tvätt-steg med vattenförhållandet 1:6 (kli:vatten).

Syftet med detta projekt var att minska vattenanvändningen i stärkelsetvätten, samt att undersöka om och i vilken utsträckning de potentiella funktionella ingredienserna åker ut i vattnet, slurryn, under denna process. Experiment utfördes med tre olika vattenförhållanden (1:6, 1:8, 1:10; kli:vatten) för svällningssteget och tre olika vattenförhållanden (1:4, 1:5, 1:6; kli:vatten) för de följande fem tvättarna.

För alla experiment minskade stärkelseinnehållet på kliet från 9,00 % till under 1,00 % stärkelse efter tre tvättsteg. Vattenmängden som behövs har begränsats från 1:10 till 1:6-1:8 (kli:vatten) för svällvatten och från 1:6 till 1:5 eller mindre (kli:vatten) för tvättvattnet, utan att påverka processens effektivitet. Den totala vattenanvändningen sänks därmed med minst 20 %. Små mängder arabinoxylaner (<0,4 mg/g slurry) och proteiner (<0,3 mg/g slurry) hittades i den vattenlösliga delen av slurryn. Minst 40% av den vattenolösliga delen av slurryn var inte stärkelse och har potential att innehålla värdefulla funktionella ingredienser. Framtida studier behöver fokusera på att undersöka vad som finns i den vattenolösliga delen av slurryn från stärkelsetvätten. Detta projekt kan leda till en minskning av miljöpåverkan och hjälpa livsmedelsindustrin att fokusera på rätt näringsämne när de planerar att avlägsna stärkelse från vetekli samt extrahera dess näringsämnen. Projektet är därmed en del av optimeringen för ett bättre utnyttjande av redan producerad mat.

Nyckelord: Fraktionering, funktionella ingredienser, stärkelse-tvätt, vetekli

Table of contents

List of tables

List of figures

Abbreviations

a/x	Arabinose-to-xylose ratio
AX	Arabinoxylan
dw	Dry weight
H/H	1:10 swell water ratio and 1:6 wash water ratio, lab scale
H/L	1:10 swell water ratio and 1:4 wash water ratio, lab scale
L//L	1:6 swell water ratio and 1:4 wash water ratio, upscaled
L/H	1:6 swell water ratio and 1:6 wash water ratio, lab scale
L/L	1:6 swell water ratio and 1:4 wash water ratio, lab scale
M//M	1:8 swell water ratio and 1:5 wash water ratio, upscaled
M/M	1:8 swell water ratio and 1:5 wash water ratio, lab scale
slurry	The water that was filtered away during the de-starching process
WB	Wheat bran
WE	Water extractable
Ws	Swelling water, the slurry from swelling in de-starching process
WUE	Water unextractable
ww	Wet weight
Ww	Wash water, the slurry from wash 1-5 in de-starching process

1 Introduction

The world is in front a complex problem: The human population continuously grows while the area for food production does not. Produced food must be utilized more efficient. Wheat is one of the most produced grains in the world, yet we sort out 15 % of the most nutritious part which is wheat bran, WB (Delcour & Hoseney, 2010). The annual world production of WB is 112 million tons (FAO, 2017). This side stream product from the milling industry is considered to be of low value, used mainly as animal feed (Schooneveld-Bergmans, M.E.F van Dijk, Y.M Beldman, G Voragen, 1999; Aguedo *et al.*, 2014).

WB contains proteins and hemicelluloses, which could provide dietary health benefits and functional properties useful in the food industry. The hemicellulose arabinoxylan (AX), present in WB, is associated with reduction of post-prandial glycaemic responses in humans, maintaining a balanced blood glucose level (EFSA, 2011; Jacquemin *et al.*, 2012). A balanced blood glucose level may be a part of a strategy to prevent the development of non-communicable diseases, which goes along with the Global Goal For Sustainable Development number 3.4, aiming to reduce in mortality from such diseases (United Nations, 2018). However, the highly viscous solution that AXs from WB forms in the stomach, seems to reduce the absorption of nutrients in monogastric animals. The nutrients get embedded into the indigestible fibre solution and passes the intestinal uptake area unaltered, which might result in nutrient deficiency (Antoniou *et al.*, 1981). Therefore, WB is currently not used to its full potential. WB proteins and hemicelluloses could instead be used as functional ingredients in other food products. Use of WB in a more efficient way is in line with the Global Goal For Sustainable Development number 12.2 which target the increase in sustainable management (United Nations, 2018).

The potentials of WB have been recognized but today most of the developed methods focus on the recovery of only one compound. To enable extraction of several compounds that are intact to a high extent, the separation methods included should be non-destructive.

An initial pre-treatment step, the de-starching of bran, is needed to simplify the extraction and purification in later steps. A non-destructive method is removal by allowing the bran to swell in water, forming a slurry, followed by several washing steps (Jacquemin *et al.*, 2012). The method requires a high amount of water to ensure that all starch is removed. A reduced use of water would be in accordance with The Global Goals For Sustainable Development number 6.4, which addresses an increase of the water use efficiency (United Nations, 2018). Research on the slurry, formed during de-starching which possibly contain desired water extractable (WE) compounds, is limited. Mild extraction methods of fibres have been developed, while those for mild protein extraction require improvement (Zhang *et al.*, 2014; Ruthes *et al.*, 2017).

There is a need to investigate if the water used for de-starching of bran could be decreased without compromising method efficiency. Knowledge on the content of the slurry is needed to evaluate if it could be further utilized. The aim of this project was therefore to optimize the de-starching process of WB. The following two aspects were considered:

- Water amount used during de-starching, through trials with different water ratios in the swelling and washing steps of the bran.
- Composition of starch slurry by chemical analysises.

2 Background

2.1 Wheat

Wheat – *Tritucum aestivum* – is one of the three most produced cereals in the world, with an annual production of 750 million tons (FAO, 2017). During milling, the wheat kernel is divided into three parts; germ (2.5-3.5 %), starchy endosperm (82-83 %) and bran (14.5-15.2 %) as seen in Figure 1 (Delcour & Hoseney, 2010). The kernel is milled through rolls and sieved several times, obtaining different purities of starchy endosperm, what we know as flour. Decades of research is lying behind the flours that make the bread, pasta or cookie to satisfy the consumer (Evers & Millar, 2002; Xie *et al.*, 2008). Chemical composition of flour is shown in Table 1.

2.1.1 Wheat bran

WB is the outer part of the wheat kernel, contributing to stability of the kernel and protection of the germ. The bran is rich in proteins, fibres and minerals (Table 1). Milled WB consists of different tissues, starting with the pericarp on the outside, followed by testa, hyaline layer and a nutritious outer part of the endosperm, called aleurone layer (Delcour & Hoseney, 2010). WB is generally not purified during milling since it is considered to be a low value product. It does therefore differ in size (>1000 µm) and in starch content (15 – 26 % in dry

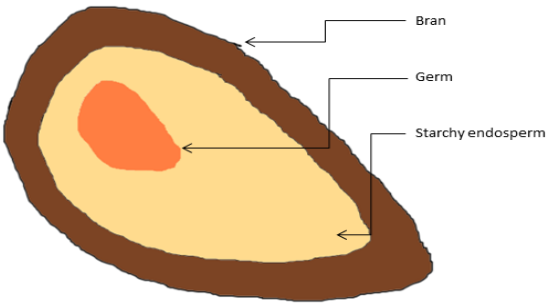

Figure 1. The milling parts of a wheat kernel; bran, germ and starchy endosperm.

weight, dw, Table 1), depending on how many times the bran passes the roller mills and sieves.

2.1.2 Wheat starch

Starch is a storage molecule for energy in wheat. For humans, starch is an important food ingredient. Starch structure is made up by 25 % amylose and 75 % amylopectin chains. Amylose are straight chains of alfa-1.4-linked glucose monomers. Amylopectin are branched due to occasional alfa-1.6-links between the glucose residues, forming the crystallin water insoluble structure of starch, called granules (Figure 2). When starch is heated together with a solution the crystalline structure opens and takes in the solution while amylose leaks out. This process is called gelatinization and gives rise to viscosity and gel properties in bread, pasta and sauce (Delcour & Hoseney, 2010). The gelatinization temperature of starch is 51-60°C in water and 20°C in alkaline solutions (Jacquemin *et al.*, 2012). Wheat starch is divided into two types of granules; bigger ones with a diameter of >10 µm and smaller ones with a diameter of <10 µm (Delcour & Hoseney, 2010). The granule types are distributed differently within the wheat kernel, giving various properties depending on what wheat part is used. WB contains 32-45 % of the small granule, compared to commercial wheat starch from flour with 15-31 % (Xie *et al.*, 2008). Smaller starch granules have potential to become fat replacers, due to a smoother mouth feel. The small granules might also be suitable for non-food application such as making plastic film fillers (Liu & Ng, 2015). Furthermore, the proportions in starch granule types also give WB a wider gelatinization temperature range, a lower viscosity at pasting peak and

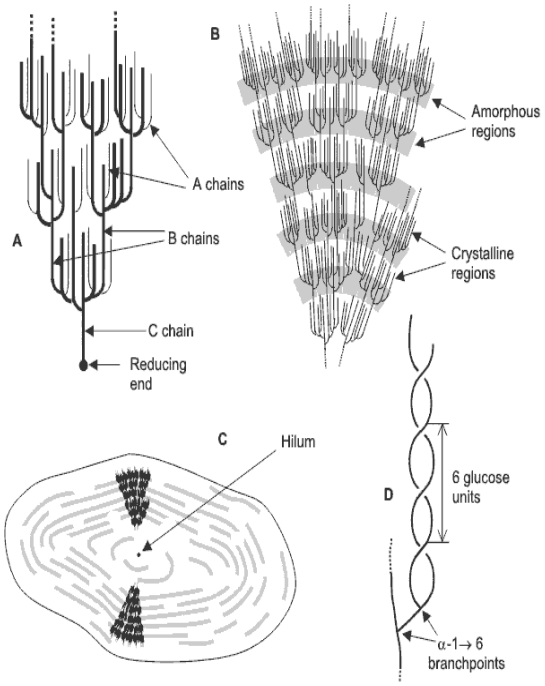

Figure 2. Amylopectin and the starch granule. (A) The essential features of the cluster model first proposed by Robin in 1974. (B) The organisation of the amorphous and crystalline regions (or domains) of the structure generating the concentric layers that contribute to the 'growth rings' that are visibly by light microscopy. (C) The orientation of the amylopectin molecules in a cross section of an idealised entire granule. (D) The likely double helix structure taken up by neighbouring chains and giving rise to the extensive degree of crystallinity in a granule. - Reproduced by permission of The Royal Society of Chemistry.

a lower retrogradation level. Those properties could be useful in the food industry in a wide range of products. For example, a lower retrogradation slow down the bread staling process, extending the shelf life.

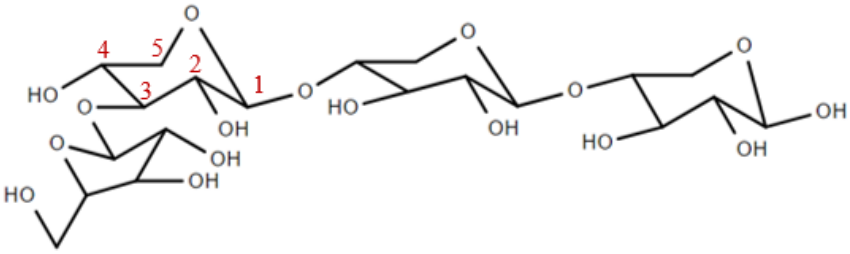

Figure 3. Part of an arabinoxylan chain, a xylose chain with an arabinose monomer on carbon number 3.

2.1.3 Wheat dietary fibres

Non-starch polysaccharides in wheat are divided into cellulose, lignin, fructans and hemicelluloses with AX (6-7 %) as the predominant one (Knudsen, 1997; Gebruers *et al.*, 2008; Delcour & Hoseney, 2010). The health claim to reduce post-prandial glycaemic responses can be made for AX (EFSA, 2011). Short AX chains also seems to have a prebiotic effect, promoting the growth of lactobacillus and bifidobacterial groups in the intestines (S. A. Hughes *et al.*, 2007). AX has a back-bone of beta-1.4-linked D-xylopyranosyl residues, substituted with monomeric alfa-L-arabinofuranose at the C(O)-3 and C(O)-2 position (Delcour & Hoseney, 2010), see Figure 3. WE-AX can form viscous solutions, where the viscosity depends on the length of the AX chains, the substitution pattern and the degree of substitution as well as cross-linking with ferulic acid. The ratio of arabinose-to-xylose (a/x) is a commonly used parameter to determine water extractability, where a high amount of arabinose seem to cross-link with other molecules and make AX less WE. However, a/x of water unextractable (WUE-) AX and WE-AX in wheat flour partly overlap, indicating that a/x is not the whole explanation for water extractability (Ordaz-Ortiz & Saulnier, 2005). WE-AX from WB have been reported to have a high a/x of around 1 (Gebruers *et al.*, 2008). Compared to WE-AX, WUE-AX instead possesses a high water holding capacity.

AX is highly distributed to the bran, seeing that refined flour only have a content of 1-2 % (Delcour & Hoseney, 2010). The AX of WB mainly consists of a variant called glucuronoarabinoxylan. The structure of glucuronoarabinoxylan is similar to the structure of AX except for incorporated glucuronic acid molecules, making the AX less water extractable (Bergmans *et al.*, 1996; Stone & Morell, 2009; Delcour & Hoseney, 2010).

A baking trial by Koegelenberg and Chimphango showed that replacing 2.5% of the flour with 0.8% of AX from WB did not change physical attributes of bread but increased the amount of dietary fibre (Koegelenberg & Chimphango, 2017). The composition of non-starch polysaccharides in WB also improved the gelling formation during cooling compared to non-starch polysaccharides in other parts of the kernel (Cui *et al.*, 1998). This further confirms the potentials of WB compounds as functional ingredients.

Fructan is an oligo- and polysaccharide of fructose units, divided into different types depending on their branching. WB mainly contain a highly branched type called graminan, with fructose chains of 3-19 monomers (Haskå *et al.*, 2008). Graminan fructans are currently invested for their anticancerogenic properties in the colon (Van den Ende, 2013).

2.1.4 Wheat proteins

Cereal proteins are usually divided into four groups based on solubility. Albumins are soluble in water, globulins in salt solutions, gliadins in 70% ethanol and glutenin in a weak acid or base (D'Ovidio *et al.*, 2009). Albumin and globulins make up 15% of the flour proteins and 39.5% of the bran proteins (Idris *et al.*, 2003; D'Ovidio *et al.*, 2009). Both albumins and globulins are relatively high in tryptophan, methionine and lysine, making them more nutritionally valuable than glutenin and gliadins (Campas-ríos *et al.*, 2012). The functional properties change with a change in protein types. Foaming and emulsifying properties are better in WB proteins which is beneficial for food formulation (Idris *et al.*, 2003). Other potential properties are prevention of enzymatic browning (Ortíz-Estrada *et al.*, 2012).

D'Ovidio *et al. (*2009) investigated how the proteins are distributed within the bran. The aleurone layer have a higher protein content (22.9 %) compared to testa (5.7 %) and pericarp (5.1 %). Additionally, the aleurone layer takes up a bigger part (7 %) of the kernel, than testa (3 %) and pericarp (5 %).

2.1.5 Wheat micronutrients and bioactive compounds

Wheat is a good source of vitamins, such as niacin, riboflavin, pantothenic acid, thiamine and pyridoxine, which are generally concentrated in the aleurone layer (Delcour & Hoseney, 2010). About 61 % of the minerals – calcium, copper, iron, magnesium, manganese, phosphorus and potassium – are present in the aleurone layer, making the WB nutritious from a trace element perspective. Sander and Andrén (1997) have seen potentials in using the ash from wheat straw as fertilizer and wheat straw have a similar ash content as WB. Wheat kernel phytochemicals

include phenolic compounds, phytosterols, alkylresorcinol, ferulic acid and flavonoids (Onipe *et al.*, 2015). Phytochemicals are plant derived molecules and many of them are known for their antioxidative, anti-inflammatory, antiallergic, anticarcinogenic and antiviral properties in humans (Youdim & Joseph, 2001). Phytosterols, present in plant membranes, have a similar structure to cholesterol, which could potentially help to keep the cholesterol level of humans in balance (Piironen *et al.*, 2000). Plant sterols have a linear relationship with higher ash content and since WB is high in ash, it becomes interesting for phytosterol extraction. Development of extraction techniques for bran compounds is in progress (Ou & Kwok, 2004; Luthria *et al.*, 2015). Extracted ferulic acid is used as substrate for vanillin production, skin protecting agent and as sport food ingredient.

Table 1. *Composition of WB and flour (dw %).*

Compound	Bran (% dw)	Flour (% dw)	Reference
Size	>1000 µm	<132 µm	(Kamal-Eldin *et al.*, 2009; Delcour & Hoseney, 2010)
Starch	8.8-26.12	80.5	(Andersson *et al.*, 1993; Dornez *et al.*, 2006; Li *et al.*, 2006; Hemery *et al.*, 2007; Kamal-Eldin *et al.*, 2009)
Dietary fibres	11.5-53.1	3.1-3.3	(Andersson *et al.*, 1993; Li *et al.*, 2006; Gebruers *et al.*, 2008; Babiker *et al.*, 2009; Van Craeyveld *et al.*, 2009; USDA, 2018)
Arabinoxylan			
- Total	12.7-30	1.93-2.1	(Andersson *et al.*, 1993; Dornez *et al.*, 2006; Gebruers *et al.*, 2008; Delcour & Hoseney, 2010)
a/x ratio	0.50-0.65	0.48-0.58	(Gebruers *et al.*, 2008; Kamal-Eldin *et al.*, 2009; Shewry *et al.*, 2013; Ruthes *et al.*, 2017)
-WE	0.3-0.85	0.51-0.8	(Andersson *et al.*, 1993; Dornez *et al.*, 2006; Gebruers *et al.*, 2008; Shewry *et al.*, 2013)
a/x ratio	0.7-1.25	0.5-0.67	(Ordaz-Ortiz & Saulnier, 2005; Gebruers *et al.*, 2008; Shewry *et al.*, 2013)
Cellulose	7.2-12.1	0.06	(Knudsen, 1997; Kamal-Eldin *et al.*, 2009; Shewry *et al.*, 2013)
B-glucan	2.15-2.6	0.64	(Knudsen, 1997; Li *et al.*, 2006; Kamal-Eldin *et al.*, 2009; Shewry *et al.*, 2013)
Fructan	2.0-3.7	0.6-1.6	(Haskå *et al.*, 2008; Kamal-Eldin *et al.*, 2009; Shewry *et al.*, 2013)
Lignin	3.3-4.9	-	(Kamal-Eldin *et al.*, 2009)
Protein	15.2-18	10.2-12.9	(Dornez *et al.*, 2006; Li *et al.*, 2006; Babiker *et al.*, 2009; Kamal-Eldin *et al.*, 2009; Delcour & Hoseney, 2010)
Lipids	3.45-5.6	0.11-1.17	(Babiker *et al.*, 2009; Kamal-Eldin *et al.*, 2009; Delcour & Hoseney, 2010; USDA, 2018)
Ash	5.5-6.5	0.4-0.61	(Andersson *et al.*, 1993; Dornez *et al.*, 2006; Babiker *et al.*, 2009; Kamal-Eldin *et al.*, 2009; Delcour & Hoseney, 2010)

2.2 Wheat bran fractionation process steps

2.2.1 De-starching of wheat bran

As stated in part 1.1.2, starch gelatinize at 20 °C in alkaline solutions and at 51-60°C in non-alkaline solutions. Therefore, a de-starching step is important to allow centrifugation and ultrafiltration as well as boiling in later steps of bran fractionation and purification. Jacquemin *et al.* (2012) and Koegelenberg & Chimphango (2017) de-starched the bran to below 1 % (dw) starch by 15 minutes of stirring in 40 °C water (10% w/w) followed by three wash steps. Aguedo *et al.* (2014) used the same water ratio but higher temperatures and shorter stirring time, decreasing the starch content to well below 5 %.

2.2.2 Extraction of proteins

The extraction rates of proteins from WB are low compared to starch and AX (D'Ovidio *et al.*, 2009). Extraction rates up to 82.5 % have been seen on grounded bran (< 400 µm) at 60-90 °C, with a pH of 12.5 and various incubation times (Roberts *et al.*, 1985; Idris *et al.*, 2003). The highest extraction rate of proteins from whole bran was 55 %, which was obtained by using 0.05 M NaOH, a temperature of 60°C and multiple extractions (De Brier *et al.*, 2015).

2.2.3 Extraction of fibres

There are several extraction methods for AX. The alkaline method, generally using NaOH for WB extraction, has extraction yields of 74-88 % (Bataillon *et al.*, 1998; Zhou *et al.*, 2010). NaOH efficiently break the cell walls by disrupting the covalent and hydrogen bonds between in the fibre structure. The treatment could also cause breakage of the ester linkages to ferulic acid in AX, and thereby disturbing the antioxidant activity (Zhou *et al.*, 2010). Zhang *et al.* (2014) reports enzymatic extraction rates of 50 %, where endo-β-1.4-xylanases cleaves the backbone of AX to xylo-oligosaccharides and WE-AX. The lower yield compared to chemical extraction could be due to natural enzyme inhibitors (Wang *et al.*, 2014). Steam explosion and microwave extraction are efficient treatments of cell walls but the degradation of AX is uncontrolled (Josefsson *et al.*, 2002; Rose & Inglett, 2010). Ultrasound is also efficient but the degree of AX degradation is not determined yet (Hollmann *et al.*, 2009; Wang *et al.*, 2014). Subcritical water extraction in combination with enzymatic treatment has shown similar extraction yields - 72.3 % - as alkaline treatment, but with intact AX structures (Ruthes *et al.*, 2017).

2.3 Methods for chemical analysis

2.3.1 Starch analysis

Starch is a well-studied compound and therefore the analysis method is well established. The official method - AOAC No 996.11 - is a procedure including two hydrolysing enzymes. In the first step, thermostable α-amylase is added to the sample, which is then heated to 100°C, hydrolysing starch into maltodextrins. Secondly, amyloglucosidase hydrolyses maltodextrin to D-glucose. Here, D-glucose can be quantified in several different ways. One method is to phosphorylate D-glucose to glucose-6-phosphate (G-6-P), oxidise G-6-P using $NADP^+$ and the concentration of NADPH is then measured in a spectrophotometer, equal to the concentration of D-glucose. (Megazyme, 2017)

2.3.2 Protein analysis

Bradford Protein Assay is a common method to measure protein concentration. It is based on a spectrometric method where Coomassie Brilliant Blue is used to change the color of the protein solution to blue. The solution is then easily measured in a spectrophotometer at 595 nm, with no or little interference of sugars in the sample. (Bradford, 1976)

2.3.3 Dietary fibre analysis

In the AOAC Method 994.13 neutral sugars, Klason lignin and uronic acids are analysed. The fibres are broken down into monomers and converted into volatile alditol acetates, which is then quantified by gas chromatography.

Nuclear magnetic resonance spectrophotometry (NMR) measures the local magnetic fields of protons through nuclei excitation of the sample (Nielsen, 2017). The magnetic field is specific for every molecule since the different attached molecules alters the signal. All the sugar molecules end up together in the spectra given, therefore NMR are mainly suitable for measuring relative concentrations of carbohydrates.

Another way to detect fibres is to add Calcofluor white stain to the sample which visualises most non-AX fibres in the microscope (Wood & Fulcher, 1983).

3 Materials and methods

3.1 Materials

Coarse WB (big flakes and a lower starch content) was subsidized by Lantmännen Cerealia. Tap water was used from Uppsala and Växjö county in Sweden. Equipment were dispensed by SLU (Swedish University of Agricultural Sciences, Ultuna) and Lantmännen Reppe (Växjö). Starch assay kit used were from Megazyme (Bray) and all other chemicals used were from Merck (Darmstadt) if nothing else is specified in the method part.

3.2 Methods

3.2.1 Experimental design

The de-starching process was adjusted compared to previous research mentioned in part 1.2.1. The process is described in Figure 5 and equipment is seen in Appendix 1. A temperature close to room temperature, 25°C, was combined with a longer swelling time, 2 hours, two additional washing steps and lowered water ratios. Three different bran:water-ratios were chosen for the swelling step, 1:6, 1:8 and 1:10, named low (L), medium (M) and high (H) respectively. Three bran:water-ratios were chosen for the washing steps, 1:4, 1:5 and 1:6, also named L, M, and H respectively. Five experiments were made by combining swell/wash ratios together, named L/L, L/H, M/M, H/L, H/H.

In this project, a two-level factorial design with a three-replicate center point was used for the lab experiments (Figure 4). A factorial design models a linear relationship between x and y as well as makes the effect of x and y easier to understand, using a minimum of experiments (Massart *et al.*, 1998a). Y represents swell water

ratio and x represents wash water ratio. The center point of the design is the only experiment made in replicates, evaluating the experimental uncertainty (Massart *et al.*, 1998b). M/M was repeated three times, named M/M1-M/M3, representing the center point. The factors in Figure 4 were set to -1 for L, 0 for M and 1 for H.

Statistical analysis of factorial design and Tukey pairwise comparison (H, M and L) were performed by Minitab® 18.1. All calculations of regressions were made in Microsoft Excel 2016. The R^2 is a value between 0 and 1, where a value close 1 indicates that the points in the plot are in line with the trend line and thus, a linear relationship is present. Significance level used was 95%.

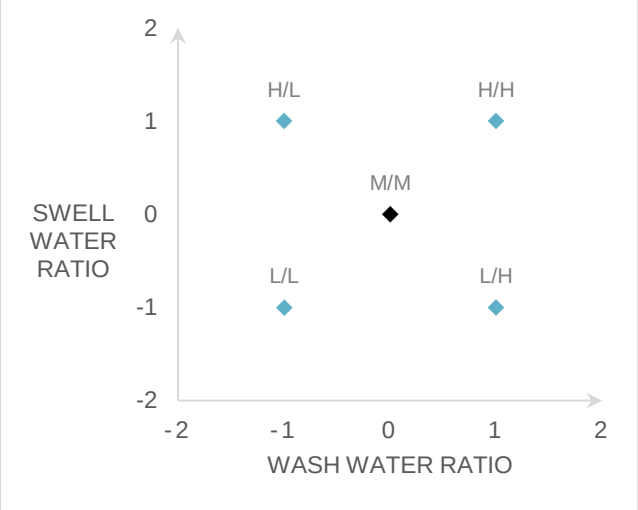

Figure 4. The two factor experimental design with a three-replicate center point (black point), showing the swell and wash ratios as y and x. -1 is equal to low, 0 to medium and 1 to high.

3.2.2 De-starching process

80 grams of WB was added to a container together with 25°C swell water (Ws). Ws was 480, 640 or 800 g for ratio 1:6, 1:8 and 1:10 respectively (Table 2). The bran solution was stirred with a magnetic stirrer for two hours. For experiments H/Hx and M/Mx, a metal container was used. The metal container required a mix speed of 70 rpm to move the solution. For the other experiments, a glass container in a water bath was used, which kept the temperature stable. The glass container was narrow and needed a stirring rate at 150 rpm to move the whole bran solution around. After stirring, the bran was filtered and gently squeezed through a metallic sieve of 600 µm until only drops came out. The whole slurry was collected in a bottle. The bran was then washed five times by adding 25°C wash water (Ww). Ww was 320, 400, 460 g for ratio 1:4, 1:5 and 1:6 respectively (Table 2). The solution

stirred another 10 minutes before each of the five Ww (Ww1-Ww5) was collected into a bottle each.

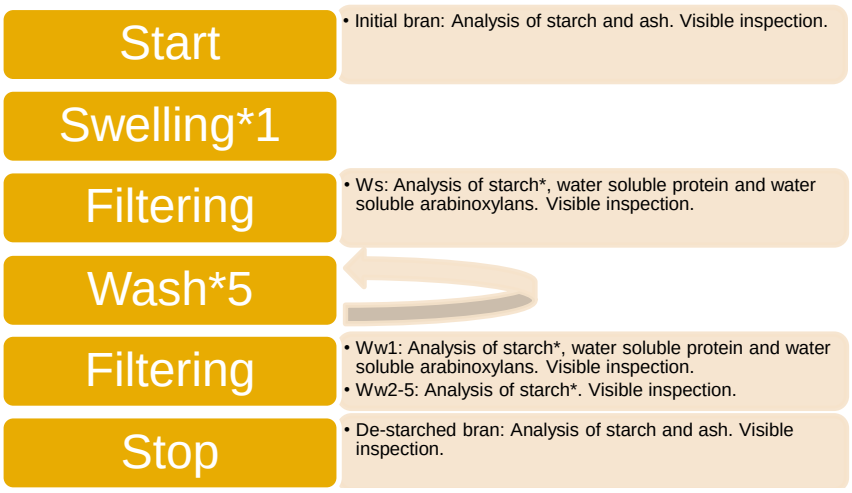

Figure 5. Left: The de-starching process with one swelling step and five washing steps of the bran. Right: Analysis schedule of bran, swell water (Ws) and wash water (Ww1-5). *Starch analysis made on selected samples.

Table 2. *The concentration of bran (g) and water (g) used in the experiments. Within in dotted line, the same water ratios were used. H stands for high ratio, M for medium and L for low.*

Experiment Name	Bran (g)	Swell ratio	Water (g) =Ws	Wash ratio	Water (g)=Ww	Stir speed (rpm)
H/Hx*	80	1:10	800	1:6	480	70
H/H						150
H/L	80	1:10	800	1:4	320	150
M/Mx*	80	1:8	640	1:5	400	70
M/Mxx*						150
M/M1						
M/M2						
M/M3						
L/H	80	1:6	480	1:6	480	150
L/L	80	1:6	480	1:4	320	150

* Experiment used for finding a proper stirring speed. These were not further investigated and are not part of the chemical compound analysis.

Data about the temperatures before and after the process steps, as well as characteristics of the slurry, were documented. Based on the visual findings from the first three experiments (marked with x in Table 2), the following experiments were adapted to obtain a consistent process.

3.2.3 Slurry sampling and visual investigation

The de-starched bran was weighted, freeze dried and then weighted again. Three of the experiments – H/Hx, M/Mx and M/Mxx - were observed during 20 hours. For the other experiments, homogenous samples were collected and frozen at -20°C for analysis of protein and fibres. Only Ws and Ww1 was sampled since it was assumed that the other wash waters would have a very low concentration of compounds. For M/M1 and M/M2, the slurry pellet was collected from all bottles and freeze-dried for starch analysis.

After 20 hours, the supernatant layer of M/Mxx had separated into two layers which was then studied in a microscope. Light microscopy was used to get an overall picture of the sample, polarized light to detect the crystalline starch structure and calcofluor white stain was used to detect non-AX fibres in the sample. After the microscopy of the two layers from M/Mxx, it was decided not to continue the investigation of layers.

3.2.4 Upscaled trials

Experiment L/L and M/M were selected for upscaled trials, named L//L1-2 and M//M1-3 (Table 3). A total of 20 kg water and bran was added into a 40 L metallic container, equipped with a stirrer that reached throughout the whole beaker, kept at 100 rpm. The procedure was in accordance with the lab scale trials, except that only four washes were made and that the slurry was not relatively quantified. Samples from Ws and Ww1, as well as initial and dried de-starched bran (overnight at 80°C), was saved for analysis. The bottles were then kept overnight to be observed after 20 hours.

Table 3. *The amount of water (kg) and bran (kg) used in the upscaled experiments. The same water ratios were used within in dotted line. M stands for medium and L for low.*

Experiment Name	Bran (kg)	Swell ratio	Water (kg) =Ws	Wash ratio	Water (kg)= Ww	Stir speed (rpm)
M//M1 M//M2 M//M3	2.2	1:8	17.8	1:5	11	100
L//L1 L//L2	2.9	1:6	17.1	1:4	11.6	100

3.2.5 Chemical analysis

Starch was determined using a total starch assay kit (Megazyme, Ireland). Approximately 80 mg of freeze dried SP sample or 100 mg milled bran was mixed with 0.2

mL ethanol (80% v/v; Solveco, Sweden) and 3 mL thermostable α-amylase (diluted 1:30 with 100 mM sodium acetate buffer, pH 5.0, with 0,74 g calcium chloride/L) in a glass test tube. The sample was incubated in a boiling water bath for 6 minutes and vigorously stirred every second minute. 0.2 mL amyloglucosidase (20 U) was added and then the tube was kept in a 50°C water bath for another 30 minutes. The sample was centrifuged (2000G, 10 minute) and diluted with deionized water (DF=33 for slurry, DF=3.3 for bran). 2 mL sample, 0.05 mL sample, 0.1 mL buffer and 0.1 mL $NADP^+$/ATP solution were pipetted into a plastic cuvette and mixed before the absorbance (A1) was measured at 340 nm. 0.02 mL hexokinase plus glucose-6-phosphate dehydrogenase suspension was mixed into the cuvette and the absorbance (A2) was measured at 340 nm every five minute until A2 was stable. A well-established control with barley starch (provided by the Department of Molecular Science, SLU, Ultuna), a blank and a D-glucose standard was also prepared and measured. The concentration of starch was calculated using the equations provided in the protocol (Appendix 2).

The concentration of protein in the slurry supernatant, was determined using the Bradford method (1978). Samples were thawed in room temperature and vortexed before used. Bradford reagent was prepared by dissolving 100 mg Coomassie Brilliant Blue G-250 in 50 ml 95% ethanol. 50 ml 85 % (w/v) phosphoric acid was added and then the solution was diluted to 1000 ml using deionized water. 100 µL sample was pipetted into a test tube with 5 mL of reagent and vortexed. The solution was incubated for 2 min, pipetted into a plastic cuvette and measured at 595 nm. If the absorbance was above the BSA standard, the 100 µL sample was diluted with tap water. Tap water was used as blank. Calculations though beer law with the extinction coefficient 6.6 was made (Appendix 2). Bovine Serum Albumin, BSA, standard solution was prepared by dissolving 100 mg of BSA into 100 ml 0.15 M NaCl. The standards were diluted to 0, 0.2, 0.4, 0.6, 0.8 and 1.0 g/mL. 1.0 g/mL BSA was additionally measured at UV 280 nm in a quartz cuvette.

Arabinoxylan in the supernatant was determined using the method for determination of soluble fibres according to AOAC Method 994.13. 7.9 mL thawed, centrifuged (5000G, 15 minutes) sample was mixed with 0.30 mL 12 M sulphuric acid and 0.50 mL 0.2% myo-inositol solution. The sample was covered with aluminum foil and hydrolyzed in autoclave at 125°C for one hour. The samples were cooled, diluted to a total volume of 10 mL, mixed and then 1.0 mL was transferred to a new test tube together with 200 µL 12 M ammonium hydroxide. pH was tested to ensure that the sample was alkaline. 100 µL 3 M potassium borohydride solution (150 mg KBH4, 250 µL 12 M ammonium hydroxide, 750µL water) was added and the test tube was incubated for 1 hour at 40°C for reduction of sugars. 100 µL glacial acetic acid was added to stop the reduction and then 500 µL sample was transferred to a

new test tube together with 500 µL 1-methylimidazole and 5.0 mL acetic acid anhydride. The tubes were mixed and incubated at room temperature for 10 minutes for sugar acetylation. 1.0 mL ethanol was added and the tubes were left for another 10 minutes. The tubes were then places in a cold water bath meanwhile 5.0 mL water and two times 5.0 mL 7.5 M potassium hydroxide was added. The upper layer was transferred to a test tube containing sodium sulfate which bound any leftover water and then the supernatant was decanted into a vial. The samples were run in a Hewlett Packard 6890 series GC system, equipped with 7683 series injector, flame-ionization detector and 7683 series auto sampler. Helium was used as carrier gas (0.8 m/s) in a program at 160 °C (6 min initial) to 220 °C (4 min final) with a rate at 4 °C/min. Calculations was made according to the protocol (Appendix 2). The amount of arabinose in AX was determined by adding xylose and arabinose after subtracting the amount of arabinose from arabinogalactan by the assumption that the arabinose-to-galactose ratio is 0.69 (Loosveld *et al.*, 1997).

The total relative carbohydrate concentration in the supernatant was determined using NMR. 450 µL centrifuged (5000G, 15 minutes) sample was mixed with 50 µL D2O (Cortechnet, France) and were then run in a Bruker Avance III 600MHz spectrometer equipped with a smartprobe. The "zgesp" pulse sequence from the Bruker standard library of pulse programs were used allowing removal of the water signal. For each spectra, 32 scans of 32k data points were collected with a relaxation delay of 4s. The spectra were then processed in TopSpin 3.1. 3-trimethylsilylpropionic acid sodium (TMSP) was added as internal standard. The calculations were based on the AX composition according to Sun *et al.* (2011) and can be seen in Appendix 2.

Ash content was determined using the method from AOAC Method 994.13 with some modifications. One gram of dry bran was placed in a pre-weighed, pre-burned crucible. The sample was burnt in 600 °C instead of 500 °C and until the samples had a constant weight instead of one hour.

4 Results

4.1 De-starching process

4.1.1 Water output

Of all the experiments, the water levels of L/H and H/L stand out. In L/H, Ws was lower than in Ww, while Ws was higher than Ww in L/H (Figures 6 and 8). The water levels were similar in Ws and Ww for H/H, M/M and L/L.

4.1.2 De-starching process operability

For most experiments the slurry passed the sieve faster, decreased in color and decreased in pellet size for every wash (Figures 6 and 8). The pellet was visible within 10 minutes after swelling and had some dark spots of bran material (Figure 7). The experiments standing out, having the same size of pellet in Ws and Ww1, were L/H, L/L and the ones with a stirring rate at 70 rpm. Additionally, Ws with ratio 1:6 were too thick to leave the treatment container without assistance.

The de-starched bran from all the lab scale experiments had a moisture content of 78 %. The dry weight of the bran after de-starching was almost halved from 75.5 g to 41.4-42.7 g (Table 4).

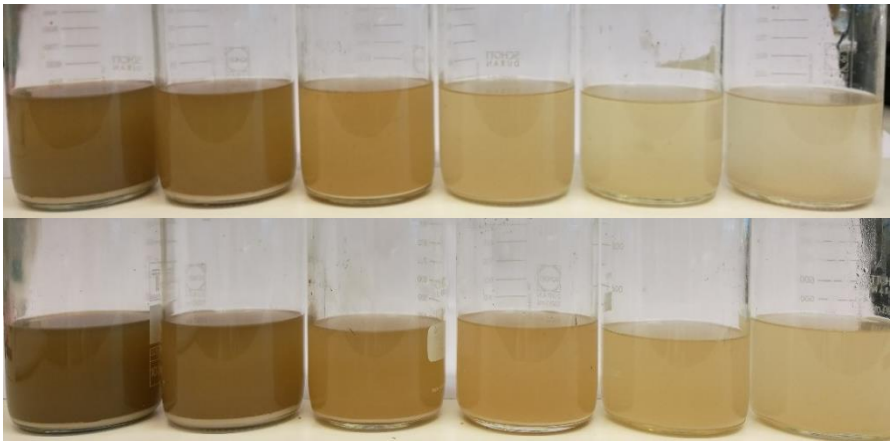

Figure 6. The lab scale slurries with a stirring rate at 70 rpm, 220 minutes after swelling. H/Hx (top) and M/Mx (bottom), From the left: Ws, Ww1, Ww2, Ww3, Ww4, Ww5.

4.1.3 Visual inspection of slurry

After 20 hours, the slurry from M/Mxx showed separation of the supernatant (Figure 7). The top layer (Figure 7, black marker) was darker than the middle layer (Figure 7, white arrow). In the light microscope, the two layers visually looked the same (Appendix 3). Both layers had rod shaped cells with movement typical for bacteria. There were also round shaped cells in a size typical for yeast. Nothing was observed in the microscope when using polarized light and calcofluor stain.

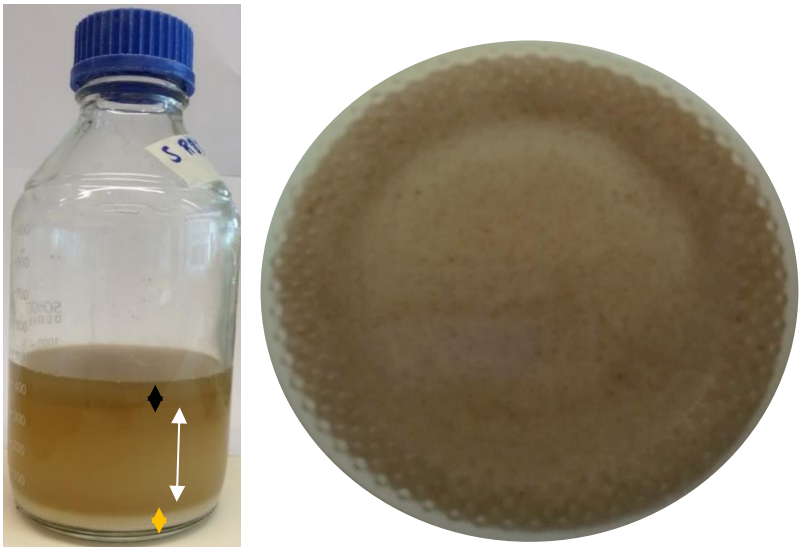

Figure 7. Left: Ws of M/Mxx after 20 hours, uncentrifuged. The yellow marker points out the pellet, the white arrow the bottom supernateant layer and the black marker the top supernatant layer. Right: Bran parts, seen as dark spots on the bottom of Ws of M/M2, representative for all Ws samples.

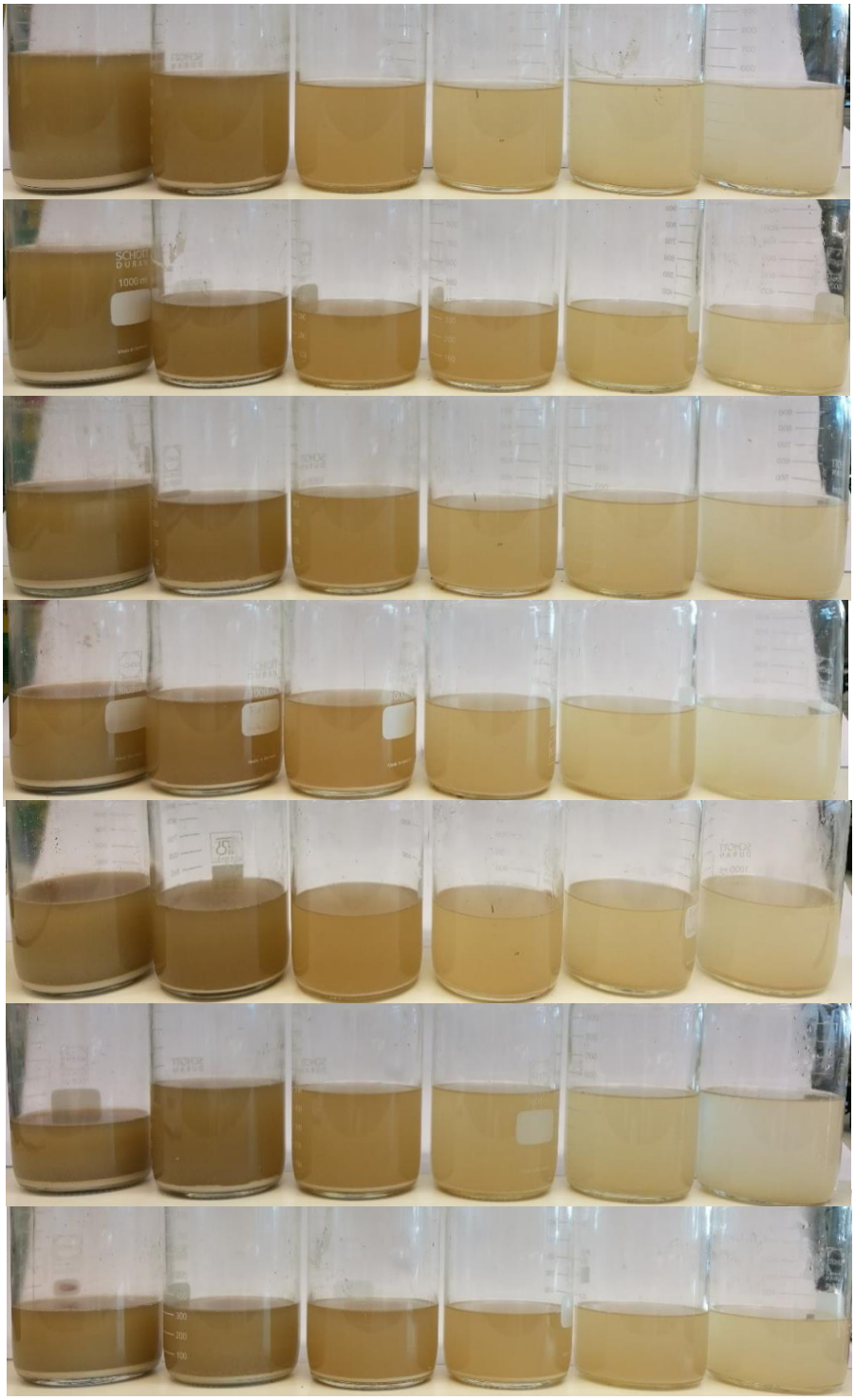

Figure 8. The lab scale slurries with a stirring rate at 150 rpm, 220 minutes after swelling. From the top: H/H, H/L, M/M1, M/M2, M/M3, L/H and L/L. From the left: Ws, Ww1, Ww2, Ww3, Ww4, Ww5.

4.2 Upscaled de-starching experiments

The slurries from the upscaled experiments are shown in Figure 9. All samples had a larger pellet in Ws than in Ww1. The color was the same between all experiments, except for Ww4 that were clearer in all M/M experiment. There was no separation of the supernatant layer after 20 hours.

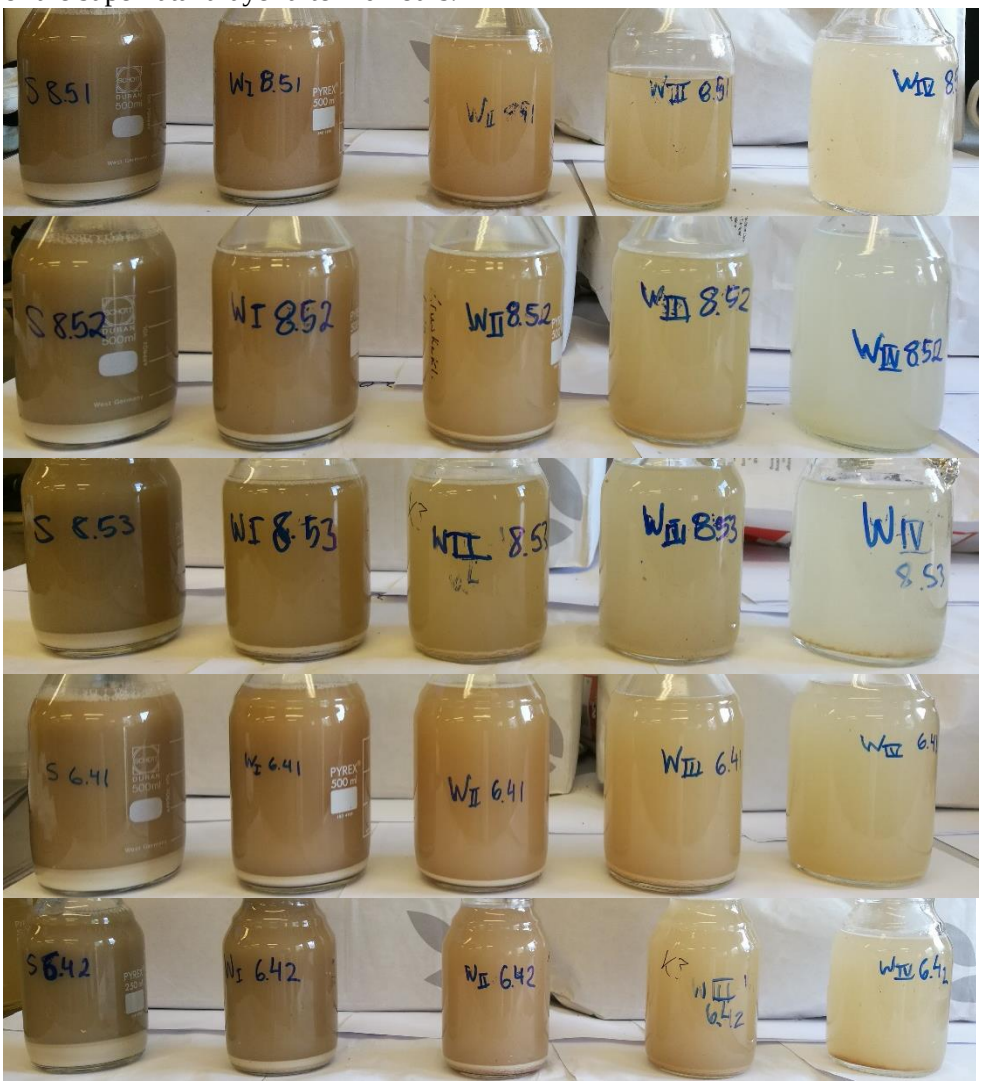

Figure 9. The slurries of the upscaled experiments, 220 minutes after swelling. From top to bottom: M/M1, M/M2, M/M3, L/L1, L/L2. From the left: Ws, Ww1, Ww2, Ww3, Ww4.

4.3 Chemical analysis

4.3.1 Starch

The starch content in the initial and the de-starched bran is shown in Table 4. The de-starching process decreased the starch content to below 1% for the lab scale experiments and to 1.2-1.5 % for the upscaled. There was no effect on starch between Ws and Ww ratios, but there was a tendency to interaction between Ws and Ww ratio (P=0.055), with an effect of 0.1003 % on starch (Figure 10 and Appendix 4). Tukey pairwise comparison confirmed the absence of significance between starch and Ws ratio (Appendix 4).

Table 4. *The weight (g) of initial and de-starched bran, as well as content of starch and ash (% of bran dw) in the bran. Analyses were made in duplicates. Standard deviation is present for ratios with duplicates of experiments.*

Experiment	Starch (%)	Ash (%)	Weight after drying (g)
Initial	9.00 ±0.009	5.6 ±0.002	76
Lab scale			
L/L	0.64	5.5	43
L/H	0.59	5.6	42
M/M	0.62 ±0.0003	5.4 ±0.002	43 ±0.1
H/L	0.59	5.1	43
H/H	0.73	5.4	41
Upscaled			
L//L	1.5 ±0.001	4.5 ±0.008	-
M//M	1.2 ±0.0009	4.2 ±0.002	-

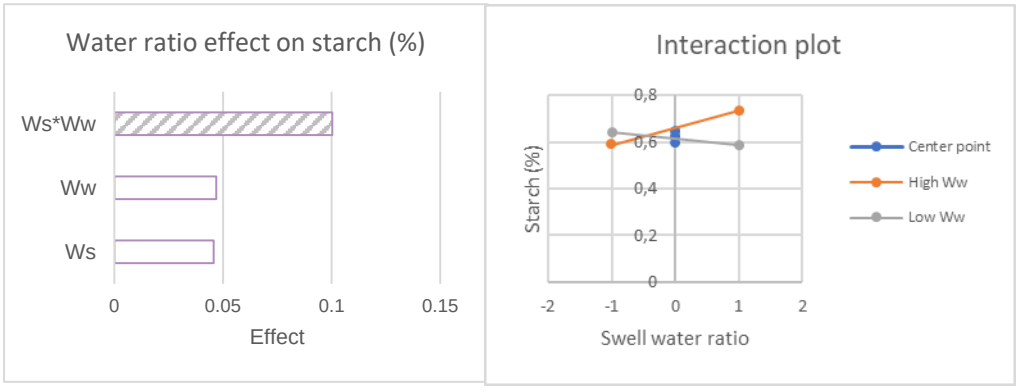

Figure 10. Left: Water ratio effects on starch (%) in de-starched bran. Grey bars are significant ($p<0.05$), white bars are insignificant ($p>0.05$) and striped bars are tending to be significant. Right: Interaction plot for the tending significant ($p=0.055$) interaction between Ws and Ww ratio, giving a 0.1 % effect on starch.

80 % of the pellet starch were distributed in Ws and the starch content decreased for every wash, going below <1% at Ww3 (Table 5). The content of starch in the pellet decreased from 62 % in Ws to 21 % in Ww5 (Table 5).

Table 5. *Relative starch distribution (%) and starch concentration (%) of M/M slurries. Analysis were made in duplicates. Standard error values are present for ratios with duplicates of experiments.*

Slurry sample	Starch distribution (%)	Starch in SP (%)
Ws	80 ±0.04	62 ±0.03
Ww1	15 ±0.005	58 ±0.008
Ww2	3.9 ±0.006	49 ±0.02
Ww3	0.88 ±0.004	31 ±0.008
Ww4	0.43 ±0.001	24 ±0.01
Ww5	0.36 ±0.004	21 ±0.03
% of total starch	74 ±0.009	

4.3.2 Protein

The protein concentrations of Ws and Ww1 are summarized in Table 6. For all experiments, the lowest protein concentrations were found in ratio 1:10, while the highest were found in ratio 1:6. The protein results showed a significant curvature between water ratios when run in Minitab and could therefore not be evaluated with the designed factorial model chosen. What can be seen is that Ws had a higher concentration of protein, 0.14-0.26 mg/g bran, than Ww1, 0.04-0.09 mg/g bran, for both lab scale and upscaled experiments. It is also noticed that when the higher concentration of protein in Ws coincided with higher in Ww1 (Figure 12), thus there was a positive relationship between these two variables (R^2=0.75).

Table 6. *Summary of compound analysis of slurries (mg/g bran dw). Analyses were made in duplicates. Standard deviation is present for ratios with duplicates of experiments.*

Experiment	Water part	Bran:Water ratio	Protein (mg/g bran)	AX (mg/g bran)[a]	Ratio A/X	Glucose (mg/g bran)
Lab scale						
L/L	S	1:6	0.24	0.26	0.53	1.8
L/H	S	1:6	0.23	0.24	0.57	1.7
M/M	S	1:8	0.16 ±0.01	0.19 ±0.009	0.55 ±0.02	1.4 ±0.03
H/L	S	1:10	0.13	0.19	0.51	1.4
H/H	S	1:10	0.14	0.18	0.53	1.4
L/L	WI	1:4	0.071	0.17	0.51	1.1
L/H	WI	1:6	0.061	0.14	0.53	0.97
M/M	WI	1:5	0.055 ±0.02	0.12 ±0.01	0.56 ±0.01	0.90 ±0.03
H/L	WI	1:4	0.051	0.12	0.51	0.86

Experiment	Water part	Bran:Water ratio	Protein (mg/g bran)	AX (mg/g bran)[a]	Ratio A/X	Glucose (mg/g bran)
H/H	WI	1:6	0.041	0.11	0.50	0.74
Upscaled						
L//L	S	1:6	0.26 ±0.004	0.25 ±0.04	0.47 ±0.21	1.7 ±0.1
M//M	S	1:8	0.17 ±0.02	0.19±0.0008	0.41±0.07	1.4 ±0.2
L//L	WI	1:4	0.085 ±0.008	0.19 ±0.04	0.47 ±0.21	1.3 ±0.1
M//M	WI	1:5	0.056 ±0.02	0.14 ±0.1	0.46 ±0.1	0.92 ±0.03

[a] Calculations of AX were made from the arabinose left after subtraction of arabinose in arabinogalactan using an arabinose-to-galactose ratio of 0.69 given by Loosveld *et al.* (1997).

4.3.3 Fibre

The AOAC Method 994.13 detected more than just AX (Table 4). Small concentrations of galactose and mannose (<0.1 mg/g bran) was found and the concentration of glucose (0.74-1.8 mg/g bran) were six to ten times higher than AX (0.11-0.26 mg/g bran). For both lab scale and upscaled experiments, the lowest AX concentrations were found in the highest water ratio, while the highest percentage were found the lowest water ratio. Ws had concentrations ranging from 0.18 to 0.26 mg/g bran and Ww1 from 0.011 to 0.19 mg/g bran, with a/x at 0.5-0.57 for lab scale experiments and 0.41-0.47 for upscaled.

The NMR spectra caught all bran fibres in one lump of sugar monomers. Peaks typical for xylose and arabinose in AX were covered by peaks matching fructose. The peaks in Ws were approximately 2 times bigger than Ww1 for all samples. A representative sample for all experiments can be seen in Figure 11.

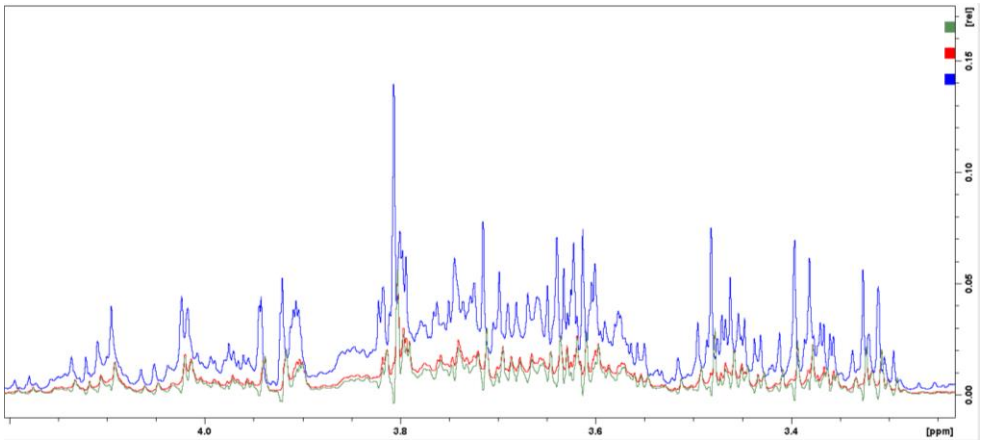

Figure 11. NMR spectra on the fibre monomers found in Ws (blue) and Ww1 (red) of M/M2. The difference in concentration between Ws and Ww1 can be seen in green color.

Between experiments the factorial model had a lack of fit for AX, glucose and a/x results, and no evaluation of water ratio impact were possible with the chosen design. Within experiments, there were positive correlations between the concentrations in Ws and Ww1 (Figure 12). The correlations were high for glucose (R^2=0.83-0.97) and protein (R2=0.75-0.88), and moderate for AX (R^2=0.65) in lab scale. For the upscaled experiment, there were positive correlations with Ws and Ww1 for all analysed compounds in the supernatant (R^2=0.83-0.99). A/x are correlated in upscaled (R^2=0.99) but not in lab scale (R^2=0.1).

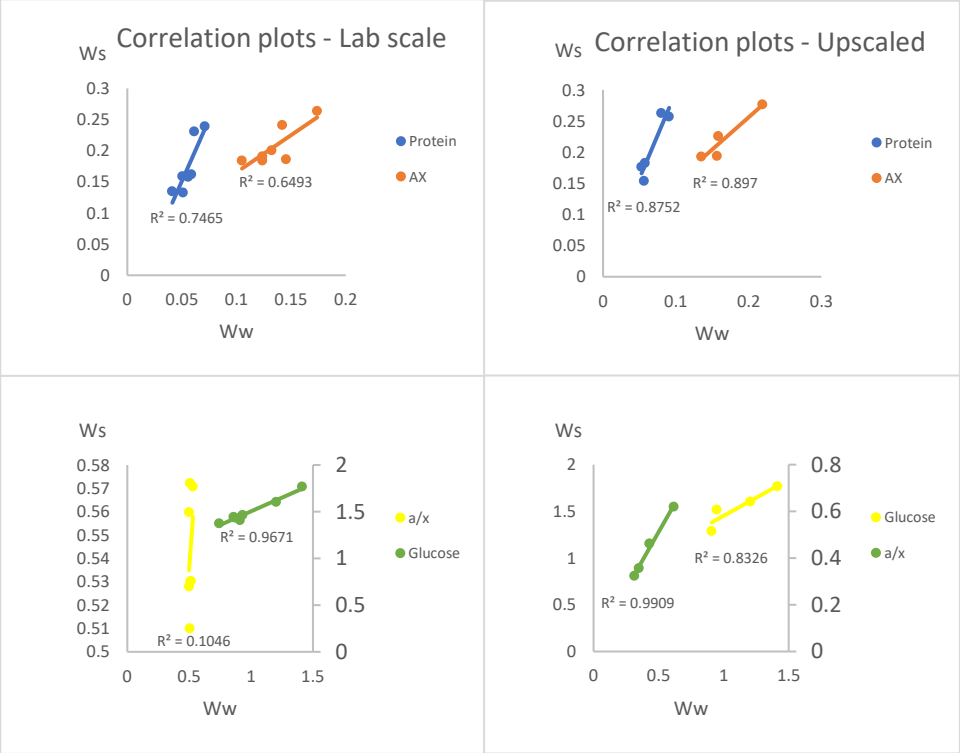

Figure 12. Correlation plots and R^2 values between concentrations (mg/ g bran) in Ws (y) and Ww (x) for protein, AX, a/x and glucose. Left column: Lab scale experiments. Right column: Upscaled experiments.

4.3.4 Slurry supernatant water extractability

By multiplying the concentrations with their dilution factor, the differences in compound water extractability could be appreciated (Table 7). In these calculations it was assumed that all WE compounds were solubilized during the swelling step of the process and that the water absorbed by brans had the same concentration of compounds as the slurry. The concentrations of WE compounds were higher when the swell water ratio was higher for glucose (R^2=0.91) and AX (R^2=0.64), (Figure 13). Tukey pairwise comparison showed that the concentration of AX and protein

differed significantly between a high and a low swelling water ratio while a/x was not (Table 8 and Appendix 4).

Table 7. *Theoretical calculations of the total WE protein, AX and glucose (% of total bran) solubilized for different water ratios used during the de-starching process. Standard deviation is present for ratios with duplicates of experiments.*

Experiment	Water part	Bran:Water ratio[b]	Protein (%)	AX (%)	Glucose (%)
Lab scale					
L/L	S	1:6	0.14	0.16	1.1
L/H	S	1:6	0.14	0.14	1.0
M/M	S	1:8	0.13 ±0.008	0.15 ±0.007	1.1 ±0.02
H/L	S	1:10	0.13	0.19	1.4
H/H	S	1:10	0.14	0.18	1.4
Upscaled					
L//L	S	1:6	0.16 ±0.0024	0.15 ±0.03	1.0 ±0.06
M//M	S	1:8	0.14 ±0.016	0.15±0.0004	1.1 ±0.16

[b] The ratio used when calculating the theoretical total concentration of WE compounds available during the de-starching process.

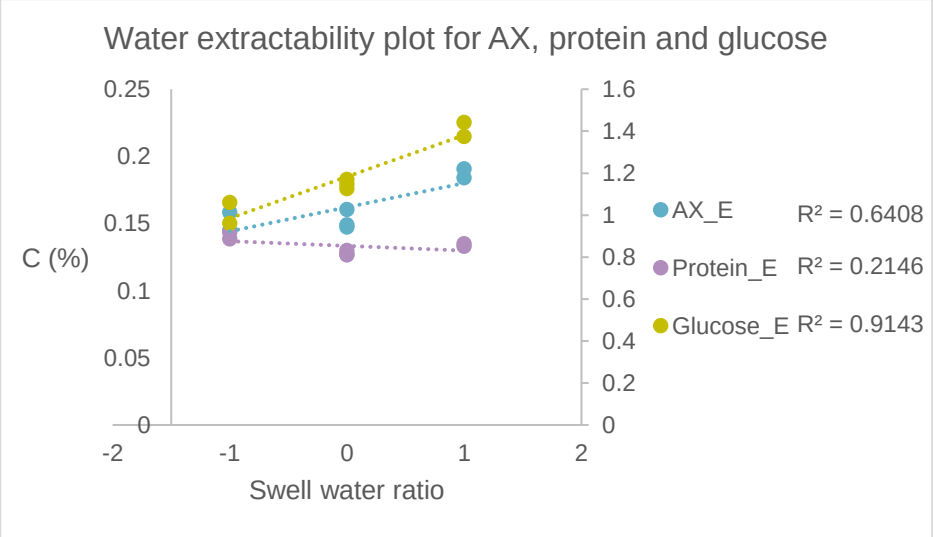

Figure 13. A plot of variations in water extractability of AX, protein and glucose changes with swell water ratio. -1 is equal to low, 0 to medium and 1 to high.

Table 8. *Tukey pairwise comparison: Different letters indicate significant effect of swelling water ratio. From the left: Protein, AX and glucose. All numbers given from Table 7, the estimated solubilized concentrations. To the right: a/x.*

Ws ratio	Protein (%)		AX (%)		Glucose (%)		a/x
H	A		A		A		A
M	A	B		B		B	A
L		B		B		B	A

4.3.5 Slurry supernatant yield

To facilitate the comparison of the supernatant analysis results with bran concentrations in dw from Table 1, the concentrations from supernatant was estimated in dw (Figure 14, raw data in Appendix 5). In these calculations it was assumed that the bran absorbed 300 % water of its weight during the swell step. Figure 15 shows correlations between concentrations and protein, glucose or AX respectively for both Ws and Ww1. Ws seemed to have a correlation with concentration in all cases (R^2=0.94-0.98). No correlations were observed in Ww1 (R^2=0.26-0.35).

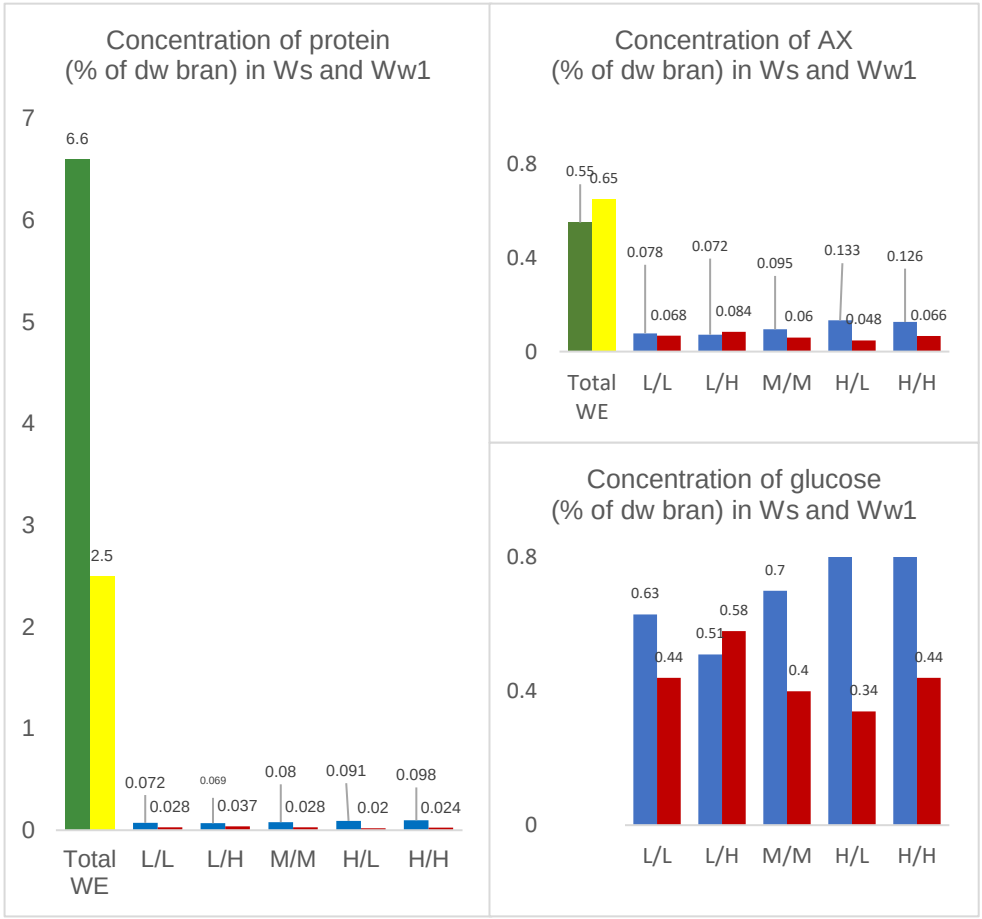

Figure 14. Estimated protein (left), AX (top right) and glucose (bottom right) leaving the bran to Ws and Ww1 (% of total dw bran). Green bars show the WE concentration in the WB, yellow bars show the WE concentration in wheat flour, blue bars show the concentration found in Ws and red bars show the concentration in Ww1.

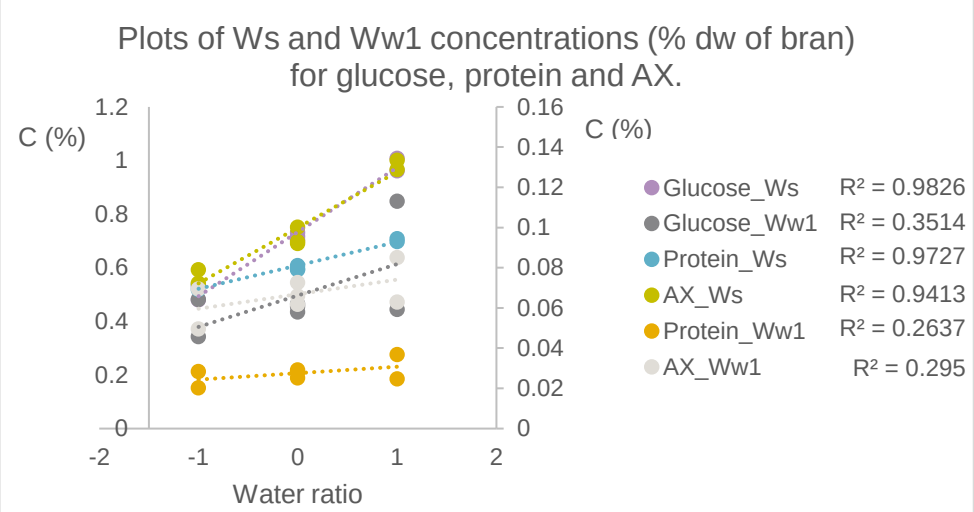

Figure 15. Plots and R^2 values showing the yield (% of dw bran) for AX, protein and glucose with swell water ratio and wash water ratio. -1 is equal to low, 0 to medium and 1 to high.

4.3.6 Ash

Ash content (% dw of bran) was 5.1-5.6 % for lab scale experiments and 4.2-4.5 % for upscaled (Table 4). Neither Ws nor Ww1 had a significant effect on ash content (Appendix 4).

5 Discussion

5.1 De-starching process in lab scale experiments

5.1.1 Experimental design

Experimental design used in the present study was dependent on results with a linear relationship and therefore inflexible. The design fitted for the concentration of compounds in bran, but not for the supernatant. The low standard deviation on M/M excludes that the observed curvatures was due to failure in the analyses. This would mean that there is no linear relationship between the slurry compounds. Since the concentrations of compounds in the supernatant were very low, the chemical compounds would probably not be further extracted. Therefore, it does not matter if the model didn't work and that we don't know what ratio is the better in this perspective. As Massart (1997) suggested, data from several experiments is needed to enable other statistical tools for investigation of possible water ratio relationships in the supernatant.

Choice of chemical compound analysis on only Ws and Ww1 were rational and time- and resources-saving.

5.1.2 Water uptake

All brans had a similar water content after de-starching, demonstrating a consistency throughout the process of the lab scale experiments. Ws was partly absorbed by the bran, explaining why the water level was similar in Ws and Ww even though more water was added in Ws. Since the water level of Ws and Ww was the same in all three M/M, it was assumed that the bran always absorbed 3 times its weight. A water absorption of 300 % for WB is consistent with Caprez *et al.* (1986).

5.1.3 De-starching process operability

For all experiments, only small spots of bran material passed the sieve, indicating that a 600 µm net was a good size for filtration. What did differ between experiments, was that a higher stirring rate gave a more efficient de-starching process. An exception was the experiments with a swelling ratio of 1:6, showing a smaller size of pellet in Ws compared to the other experiments. Another limiting factor of water ratio 1:6 for swelling was that the bran was hard to remove from the container used during the de-starching. Ratio 1:6 thereby defines a lower limit for an improved swell water ratio.

5.1.4 Visual inspection of slurry

The starch slurries dropped in colour and pellet size, which is due to lower concentration of chemical compounds, confirmed in Table 5.

The microbes observed in the light microscopy in sample M/M2 indicated that there has been bacterial and yeast growth in the slurry. This was expected given that the slurries contained glucose which is a substrate for most yeast and bacteria. To prevent bacterial growth that could spoil the slurry, the slurry should be further processed as soon as possible after the de-starching process. The two layers of supernatant seen in Ws of M/M2 (Figure 7), was due to size sedimentation, partly from bacterial degradation, rather than chemical compounds separation since there was no separation after centrifugation of the slurry. This agrees with the low concentrations observed in the results of chemical compound analysis.

5.1.5 Slurry supernatant water extractability

It is logical that higher water ratios have a lower concentration of compounds since the compounds are more diluted (Table 6). However, when compensating for the dilution degree in Table 7, we could see that the concentration order turns up-side-down. Osmosis is the reason for more solubilized WE-AX and glucose when the Ws ratio increases (Figure 13). Osmosis is also the reason that a higher concentration of chemicals in Ws gives a higher concentration in Ww (Figure 12), seeing that more particles were left in the bran after swelling.

The Tukey pairwise comparison showed significant differences in compounds being solubilized when using different swell ratio. For a/x, no significant difference was seen, indicating that any ratio can be used for the same outcome. For AX and protein, H differed significantly from L. However, the concentrations were very low (0.13-0.19 %) and this significance will most probably not have an impact when choosing what swelling ratio to use for the de-starching process.

5.1.6 Slurry supernatant yield

The concentration of WE-AX and protein in the slurry was very low compared to the total water extractable concentration (Figure 14), meaning that the yield was very low. An interesting thing seen in Figure 15 is that the wash water seems to have little or no impact on the compound concentrations. This means that the water amount used in the washing steps can be further decreased for an even more efficient washing.

5.1.7 Starch

The cause of a tendency to a significant ($p=0.055$) interaction between swell ratio and wash ratio on starch in de-starched bran can be seen in Figure 10. The red curve indicates that a high Ws ratio increase the starch content, but only for a high Ww ratio. This is highly unlikely since H/H should de-starch the bran the most since it uses more water in the process. Seeing that the concentrations of compounds between experiment is very close to each other, it is likely that there was a small analysis error changing the order on starch content left. Therefore, the tendency to interaction can be rejected. More data is needed to confirm any interactions.

All M/M experiments de-starched the bran to a high extent in Ww3 already, confirming that not all wash steps are necessary for de-starching of bran (Table 5). This is in line with de-starching methods at 40°C from both Jacquemin *et al.* (2012) and Koegelenberg & Chimphango (2017). However, the bran is not completely de-starched, and this paper does not investigate whether the de-starching is efficient enough for further utilization of WB.

The glucose present in the supernatant was most likely arrive from degraded starch, broken down during the swell step by naturally occurring amylases. Germination conditions is equal to the two-hour swelling step of the de-starching process. α-amylase increases several times during germination, resulting in degradation of starch into glucose (Delcour & Hoseney, 2010).

The starch in the pellets only represented 74 % (Table 5) of the initial WB starch, announcing that 26 % starch have been lost somewhere. The degraded starch in the supernatant (13 % of the initial starch content), together with the starch left in the bran (7 % of the initial starch concentration), explain where most of the missing starch from the pellets ended up. The remaining 4 % of all starch could have been lost when the supernatant was decanted from the pellet.

5.1.8 Protein

Albumins are WE proteins and should therefore be the main protein found in the supernatant of the slurry. Some salt extractable globulins could have been extracted since tap water contains some salts. Albumin represent 3-5 % of the flour proteins (D'Ovidio *et al.*, 2009). Assuming most of the albumins in the slurry arrived from the flour and not from the nearly intact bran, 0.1 % of WE protein (dw) is expected in the supernatant. This is in line with the supernatant protein results in dw, showing a total concentration of 0.10-0.12 % protein when Ws and Ww1 is added together (Figure 14).

The low concentration of protein extracted during the de-starching which is also seen in extraction methods for protein, imply that protein extraction methods need a pre-treatment step.

5.1.9 Fibre

The results from AOAC Method 994.13 show that the concentration of AX was 1.5-2 times higher in Ws compared to Ww1 (Table 6). This is likely since the results from NMR showed that the total sugar monomer concentration in Ws was two times higher than in Ww1 (Figure 11). NMR was useful as a screening for possible chemical compounds since it found that fructose is one of the main sugar compounds in the supernatant. This was not seen in the AOAC Method 994.13 analysis since it doesn't detect for fructose. Fructose arrives from fructan, which is WE because of its small molecule size. Fructans might have health benefits and could therefore have potential to be extracted for the use as functional ingredients as reported by Haskå *et al.* (2008).

The theoretical expected concentration of WE-AX from the slurry could be calculated using value from Table 1. That would give a theoretical concentration of 0.084 % dw WE-AX from the starchy endosperm in the slurry. Additionally, a small amount of WE-AX arriving from ruptured bran structure. The theoretical content of WE-AX in the supernatant should be around 0.1 % dw of bran, which is just below the calculated %, Ws+Ww1 = 0.146-0.192 %, from the results in Figure 14.

The low a/x (0.50-0.57) in the slurry confirm that most of the AX derives from the starchy endosperm, since a/x is around 1 in WB WE-AX (Gebruers *et al.*, 2008). Arabinogalactan is water extractable, which means that the molecule was expected in the supernatant (Timell, 1965).

The bran dropped almost 50 % in weight (Table 5) from 76 g to 41-43 g (dw). Some bran material was lost in the container and the sieve, around 9 % was starch and around 1 % was AX and protein. Where the rest of the bran weight was lost is in need of further investigation.

5.1.10 Ash

There was no correlation found between ash content and used water ratio in the bran (Appendix 4). Notice that the ash concentration was the same in initial and de-starched bran (Table 4). Only half of the bran weight was left after de-starching which means that also half of the ash must be left, other ways the concentration would be the double. That high concentration of ash could possibly be extracted and used as fertilizer (Sander & Andrén, 1997).

5.2 De-starching process in upscaled experiments

5.2.1 Water ratio experiments

The Ws of L//L had a bigger pellet than Ww1, which was not the case in the lab scale L/L. The upscaled experiments kept a lower speed of stirring at 100 rpm than lab scale. The stirrer was bigger and reached the brans all around in the beaker, which made the stirring more efficient. This is an additional implication that the stirring has a big effect on the de-starching process. The stirring or shaking rate for previous de-starching experiments from Aguedo (2014), Jaquemin (2012) and Koegelenberg & Chimphango (2017) were not specified. This implies that the knowledge about that the stirring rate have an impact on the efficiency of de-starching is either obvious or unknown.

5.2.2 Chemical analysis

Comparing upscaled experiment with lab scale, upscaled experiments had a slightly higher concentration of starch left (1.2-1.5 %) and lower a/x (0.41-0.47). It is likely not due to that upscaled only had four washing steps, since Ww4 in lab scale had a low starch concentration (Table 5). It is probably due to the homogeneity of the bran sample used. It could be more difficult to take a representative sample in a bigger batch of bran. All the supernatant analysis results from upscale have similar concentrations of AX in Ws and Ww1 (Figure 12), which differs from the lab scale experiments. A more consistent upscaled process should be developed to enable reliable comparisons.

5.3 The future of the de-starching process

Looking at the concentrations of AX, protein, starch and ash, it is clear that there were no big differences found between the concentrations of chemical compounds in the different experiments. It is difficult to draw reliable conclusions since to the best of my knowledge there is no available data in the literature on the slurry composition. Since the three replicates of M/M had low standard deviations, it can be assumed that all the tested water ratios can be used for de-starching. The commonly used swell water ratio of 1:10 by Jacquemin *et al.* (2012), Aguedo *et al.* (2014) and Koegelenberg & Chimphango (2017) could be decreased to 1:8, making the de-starching more economically and environmentally beneficial. The upscaled experiments showed signs of process problems with a swell water ratio of 1:6, indicating that the swell ratio should be higher than 1:6.

Due to multiple washes, the wash water ratio has a bigger impact of the total water use than swell water ratio. There were no difficulties with handling any wash water amount and no correlations between water amount used for wash and chemical analysis results (Figure 15). This makes it interesting to do trials using an even lower wash water ratio than 1:4. If the de-starching process is adjusted according to this research, the decrease in total water use could be at least 20 %.

Because of the yield of proteins and AX in the supernatant (Figure 14), it is presumably not advantageous to extract them from the slurry. Further research is needed to evaluate if the supernatant is worth processing regarding other chemical compounds, such as phytochemicals and fructans.

The pellet from Ws is rich in starch which could be purified and used in industry. If the starch granules are small, they could be more valuable for the industry than the commercial starch, used as fat replacer or plastic film fillers as suggested by both Xie *et al.* (2008) and Liu & Ng (2015). Furthermore, the pellet had a starch concentration of 21-62 %, which means that there are other WUE chemical compounds washed away from the bran. Some part of the pellet is bran parts (Figure 7) and some is WUE gluten proteins from the starchy endosperm. Further research is needed to investigate whether there are high concentrations of other valuable WUE compounds lost from the bran.

6 Conclusion

The aim of this project was to optimize the de-starching process of wheat bran by decreasing the water amount used during de-starching, as well as to investigate if and to what extent the starch slurry contains other compounds than starch.

The results from this project showed that the water ratios of H/H, H/L, M/M, L/H and L/L did not result in the differences in the concentration of protein, arabinoxylan, arabinose-to-xylose ratio, starch, ash, water extractability or yield. It is the handleability and the stirring rate of the wet WB that limits the decrease in water ratio for the de-starching process, rather than the effect on the chemical composition. The water needed for a kept efficient de-starching process has been narrowed down to a swell water ratio within 1:6 and 1:8 and a wash water ratio on 1:5 or lower. This decreases the total water use by at least 20 %.

The concentrations of protein and arabinoxylan were low in the slurry supernatant, while fructan and minerals could be present in higher concentrations. Further research should be focused on investigations whether it is beneficial to extract them from the slurry supernatant. Most starch ends up in the pellet of the swelling water which is beneficial for the purification of starch. The starch granules are possibly of a small size with desirable functional properties for the food industry. Furthermore, 40 % of the pellet content is unknown, making it relevant for further investigation.

This project revealed useful information for the industry when planning to de-starch wheat bran and extract its nutrients. It could thereby be a part of optimizing the de-starching process of wheat bran by decreasing the environmental impact and targeting the most economically efficient nutrients to extract. The findings in in this project could additionally be a part of fulfilling the Global Goal For Sustainable Development number 3.4, 6.4 and 12.2.

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Appendix 1

De-starching process equipment

Equipment for de-starching of wheat bran in lab scale. Left: Tthe glass container in a temperature controlled water bath. Right: The sieve used.

Appendix 2

Calculations of concentrations

Estimation of internal standard (TMSP) concentration for NMR analysis.
Assuming that the Ws had an AX concentration (C) of 8 %, the calculations emanated a concentration of 8 %, to be applicable for both Ws and Ww1. The molecular weight for TMSP is 172.27 g/mol while both arabinose and xylose have a molecular weight of 130.099 g/mol in AX. 8 % of the 450 µL (0,450 g) sample is $n_{AX} = m_{AX}/M_{AX} = 0{,}08 * 0.450/130.099 = 2.767 * 10^{-4} mol/mL$. That would give a concentration of $C_{AX} = n_{AX}/V_{AX} = 2.767 * 10^{-4}/0.450 = 6.149 * 10^{-4}$. Counting backwards for TMSP, $n_{TMSP} = C_{TMSP} * V_{TMSP} = 2.767 * 10^{-4} * 0.050 = 3.0746 * 10^{-5}$ and $m_{TMSP} = n_{TMSP} * M_{TMSP} = 3.0746 * 10^{-5} * 172.27 = 0.0053\ g/sample$. Then this concentration was halved to suit as internal standard for both Ws and Ww1.

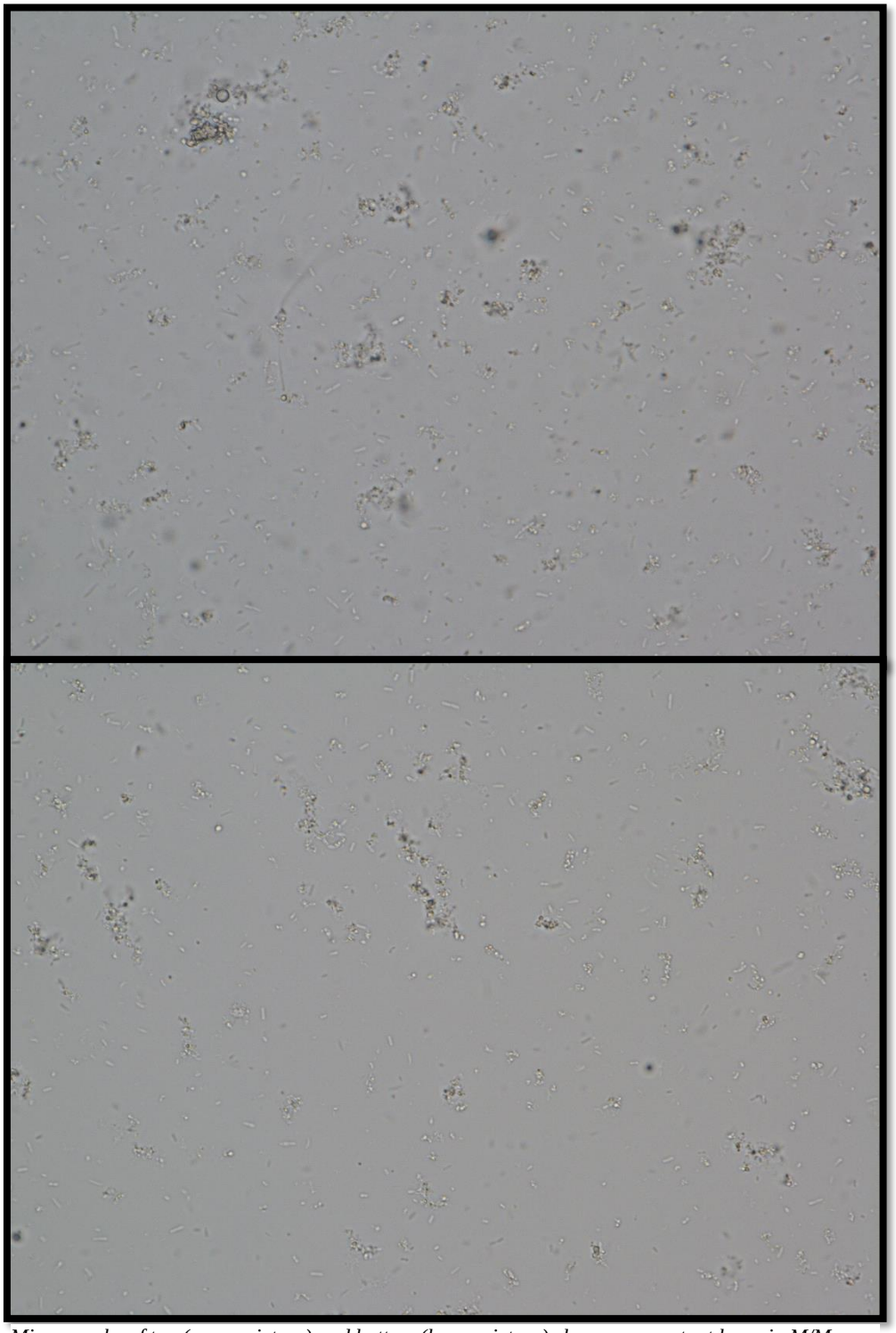
Calculations of Bradford protein method (Beer's law)
$C = A/\text{□} * b$; where C is concentration of protein, A is measured absorbance minus blank, b is path length (=1 cm) and Ɛ is extinction coefficient (= 6.6). The concentration was calculated by division of ten (g/L → %).

Calculations of WE fibre (monomers) concentration in SS
$PR = CF_m * P_m * W_s * F_m * 100/P_s * S$; where PR is the concentration of fibre, CF_m is correction factor for individual monosaccharide, P_m is peak area for internal standard, W_s is weight (mg) of internal standard, F_m is recalculation factor for individual monosaccharide to polysaccharide residues (=0.90) and S is dm for initial sample. The concentration was calculated by division of ten (g/L → %).

Calculations of starch using Starch Assay kit from Megazyme (Ireland)
$C = V * MW/\text{□} * d * v * 162/180 * \Delta A_{D-glucose}\ [g/L]$; where V is final volume (=2.27), MW is molecular weight of D-glucose (=180.16), Ɛ is extinction coefficient of NADPH at 340 nm (=6300), d is light path (=1), v is sample volume (=0.05), 162/180 is adjustment of free D-glucose to anhydro D-glucose (starch), and $\Delta A_{D\text{-}glucose}$ is A_2-A_1-blank. The concentration of the diluted samples was adjusted. The content (%) was then determined by dividing concentration with the weight of the sample.

Appendix 3

Micrographs of the slurry



Micrographs of top (upper picture) and bottom (lower picture) slurry supernatant layer in M/Mxx (Figure 7).

Appendix 4

Results from Minitab

Regression results

Factorial Regression: Starch versus Swell; Wash; CenterPt

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	4	0,014661	0,003665	6,10	0,146
Linear	2	0,004281	0,002141	3,56	0,219
Swell	1	0,002085	0,002085	3,47	0,204
Wash	1	0,002196	0,002196	3,65	0,196
2-Way Interactions	1	0,010059	0,010059	16,74	0,055
Swell*Wash	1	0,010059	0,010059	16,74	0,055
Curvature	1	0,000322	0,000322	0,54	0,540
Error	2	0,001202	0,000601		
Total	6	0,015863			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0245143	92,42%	77,27%	*

Coded Coefficients

Term	Effect	Coef	SE Coef	T-Value	P-Value	VIF
Constant		0,6362	0,0123	51,90	0,000	
Swell	0,0457	0,0228	0,0123	1,86	0,204	1,00
Wash	0,0469	0,0234	0,0123	1,91	0,196	1,00
Swell*Wash	0,1003	0,0501	0,0123	4,09	0,055	1,00
Ct Pt		-0,0137	0,0187	-0,73	0,540	1,00

Regression Equation in Uncoded Units

Starch = 0,6362 + 0,0228 Swell + 0,0234 Wash + 0,0501 Swell*Wash - 0,0137 Ct Pt

Effects Pareto for Starch

Factorial Regression: Ash versus Swell; Wash; CenterPt

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	4	0,111332	0,027833	1,52	0,433
Linear	2	0,106732	0,053366	2,92	0,255
Swell	1	0,079046	0,079046	4,33	0,173
Wash	1	0,027685	0,027685	1,52	0,343
2-Way Interactions	1	0,004493	0,004493	0,25	0,669
Swell*Wash	1	0,004493	0,004493	0,25	0,669
Curvature	1	0,000107	0,000107	0,01	0,946
Error	2	0,036519	0,018259		
Total	6	0,147850			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,135127	75,30%	25,90%	*

Coded Coefficients

Term	Effect	Coef	SE Coef	T-Value	P-Value	VIF
Constant		5,3995	0,0676	79,92	0,000	
Swell	-0,2812	-0,1406	0,0676	-2,08	0,173	1,00
Wash	0,1664	0,0832	0,0676	1,23	0,343	1,00
Swell*Wash	0,0670	0,0335	0,0676	0,50	0,669	1,00
Ct Pt		-0,008	0,103	-0,08	0,946	1,00

Regression Equation in Uncoded Units

Ash = 5,3995 - 0,1406 Swell + 0,0832 Wash + 0,0335 Swell*Wash - 0,008 Ct Pt

Effects Pareto for Ash

The regression results without curvature in lab scaled de-starched bran. Under section "Analysis of variance" P-values are seen in the right column. The only results that was tending to be significant is marked with a red box. Left: Starch. Right: Ash.

Tukey parwise comparison

One-way ANOVA: Protein_Swell versus Swell ratio

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Swell ratio	3	H, L, M

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Swell ratio	2	0.000209	0.000104	19.96	0.008
Error	4	0.000021	0.000005		
Total	6	0.000230			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0022863	90.89%	86.34%	68.51%

Means

Swell ratio	N	Mean	StDev	95% CI
H	2	0.13397	0.00146	(0.12948, 0.13846)
L	2	0.14099	0.00351	(0.13650, 0.14548)
M	3	0.12784	0.00180	(0.12417, 0.13150)

Pooled StDev = 0.00228631

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Swell ratio	N	Mean	Grouping	
L	2	0.14099	A	
H	2	0.13397	A	B
M	3	0.12784		B

Means that do not share a letter are significantly different.

One-way ANOVA: Glucose_Swell versus Swell ratio

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Swell ratio	3	H, L, M

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Swell ratio	2	0.163787	0.081893	39.98	0.002
Error	4	0.008194	0.002048		
Total	6	0.171980			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0452591	95.24%	92.85%	81.97%

Means

Swell ratio	N	Mean	StDev	95% CI
H	2	1.4079	0.0473	(1.3191, 1.4968)
L	2	1.0114	0.0704	(0.9225, 1.1002)
M	3	1.1479	0.0225	(1.0753, 1.2204)

Pooled StDev = 0.0452591

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Swell ratio	N	Mean	Grouping	
H	2	1.4079	A	
M	3	1.1479		B
L	2	1.0114		B

Means that do not share a letter are significantly different.

One-way ANOVA: AX_Swell versus Swell ratio

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Swell ratio	3	H, L, M

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Swell ratio	2	0.001795	0.000898	16.79	0.011
Error	4	0.000214	0.000053		
Total	6	0.002009			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0073111	89.36%	84.04%	66.24%

Means

Swell ratio	N	Mean	StDev	95% CI
H	2	0.18746	0.00474	(0.17310, 0.20181)
L	2	0.15158	0.00950	(0.13722, 0.16593)
M	3	0.15231	0.00711	(0.14059, 0.16403)

Pooled StDev = 0.00731111

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Swell ratio	N	Mean	Grouping	
H	2	0.18746	A	
M	3	0.15231		B
L	2	0.15158		B

Means that do not share a letter are significantly different.

One-way ANOVA: a/x versus Swell ratio

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Swell ratio	3	H, L, M

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Swell ratio	2	0.001631	0.000816	1.70	0.291
Error	4	0.001914	0.000478		
Total	6	0.003545			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0218726	46.01%	19.02%	0.00%

Means

Swell ratio	N	Mean	StDev	95% CI
H	2	0.51901	0.01268	(0.47607, 0.56195)
L	2	0.5504	0.0287	(0.5075, 0.5934)
M	3	0.5541	0.0215	(0.5190, 0.5892)

Pooled StDev = 0.0218726

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Swell ratio	N	Mean	Grouping	
M	3	0.5541	A	
L	2	0.5504	A	
H	2	0.51901	A	

Means that do not share a letter are significantly different.

Raw data for Tukey pairwise comparison between swell ratio (H, M and L) and slurry concentrations. If the ratios have the same letter, they are not significantly different. Top left: Protein. Top right: Glucose. Lower left: Arabinoxylans. Lower right: a/x.

One-way ANOVA: Starch versus Swell ratio

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Swell ratio	3	H, L, M

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Swell ratio	2	0.002407	0.001203	0.36	0.720
Error	4	0.013456	0.003364		
Total	6	0.015863			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0580009	15.17%	0.00%	0.00%

Means

Swell ratio	N	Mean	StDev	95% CI
H	2	0.6590	0.1041	(0.5451, 0.7729)
L	2	0.6133	0.0378	(0.4995, 0.7272)
M	3	0.6225	0.0245	(0.5295, 0.7154)

Pooled StDev = 0.0580009

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Swell ratio	N	Mean	Grouping
H	2	0.6590	A
M	3	0.6225	A
L	2	0.6133	A

Means that do not share a letter are significantly different.

One-way ANOVA: Ash versus Swell ratio

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Swell ratio	3	H, L, M

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Swell ratio	2	0.07915	0.03958	2.30	0.216
Error	4	0.06870	0.01717		
Total	6	0.14785			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.131051	53.54%	30.30%	0.00%

Means

Swell ratio	N	Mean	StDev	95% CI
H	2	5.259	0.165	(5.002, 5.516)
L	2	5.5400	0.0703	(5.2828, 5.7973)
M	3	5.3916	0.1351	(5.1815, 5.6017)

Pooled StDev = 0.131051

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Swell ratio	N	Mean	Grouping
L	2	5.5400	A
M	3	5.3916	A
H	2	5.259	A

Means that do not share a letter are significantly different.

Raw data for Tukey pairwise comparison between swell ratio (H, M and L) and bran concentrations. If the ratios have the same letter, they are not significantly different. Left: Starch. Right: Ash.

Appendix 5

Raw data for calculations of concentrations in % of dw bran, seen in Figure 14.

Estimated protein AX and glucose (% of total bran dw) leaving the bran in Ws and Ww1, called the yield. Standard deviation is present for ratios with duplicates of experiments.

Experiment	Water part	Bran:Water ratio[c]	Protein (%)	AX (%)	Glucose
Lab scale					
L/L	S	1:3	0.072	0.078	0.63
L/H	S	1:3	0.069	0.072	0.51
M/M	S	1:5	0.080 ±0.005	0.095 ±0.005	0.70 ±0.02
H/L	S	1:7	0.091	0.133	0.98
H/H	S	1:7	0.098	0.126	0.98
L/L	WI	1:4	0.028	0.068	0.44
L/H	WI	1:6	0.037	0.084	0.58
M/M	WI	1:5	0.028 ±0.01	0.06 ±0.005	0.40 ±0.02
H/L	WI	1:4	0.020	0.048	0.34
H/H	WI	1:6	0.024	0.066	0.44
Upscaled					
L//L	S	1:3	0.078 ±0.001	0.075 ±0.01	0.51 ±0.03
M//M	S	1:5	0.085 ±0.01	0.095±0.0004	0.70 ±0.1
L//L	WI	1:4	0.034 ±0.003	0.076 ±0.02	0.52 ±0.04
M//M	WI	1:5	0.028 ±0.01	0.070 ±0.05	0.46 ±0.02

[c]After correction of a 300% water loss in Ws due to water absorption of bran.

Appendix 6

Popular Science Abstract

The world is continuously growing while the areas for food production seems to shrink. To be able to survive, we need to optimize the utilization of already produced food. One way of doing this is to use the annual production of 112 million ton of wheat bran in a more efficient way than today. Wheat consist of is 15 % of wheat bran which is separated away in the production of white wheat flour and given to the animals. Wheat bran is the outer part of the wheat kernel, containing high contents of minerals, vitamins and phytochemicals. Phytochemicals is small molecules which could have health benefits for humans such as anti-inflammatory, anti-carcinogenic and antioxidative properties. Wheat bran also contains a lot of proteins and fibres in wheat bran with properties that can be useful in food structure modification, making the food more attractive for the consumers. Additionally, wheat fibres are known to keep the blood glucose level in balance. A balanced glucose blood level is a way to decrease mortality in non-communicable diseases. Those diseases were responsible for 68 % of the deaths in 2012 according to a status report from WHO. Wheat bran could be utilized in a more optimal way if starch, protein, fibres and phytochemicals were extracted from it.

The first step in the extraction process is to remove starch from the bran using a two-step process. In the first step one part bran is stirred in ten parts water. In the other step the bran is washed several times with six parts water. The knowledge about if the process could be made using less water, making the process more environmentally friendly, is limited. The knowledge about if the wheat bran loses anything else than starch during the de-starching process is also hard to find. Trying to decrease the water use and investigating if and to what extent other compounds than starch leaves during de-starching was the aim of this project. Trials of de-starching was made with three different water amounts (100%, -20 %, -40 %) for the swelling part and three different water amounts (100%, -17%, -33%) for five washes of the process.

Results show that all experiments decreased the starch content from 9.00 % to below 1 % in the bran after three washing steps. The optimal water ratios have been narrowed down to a decrease between 20 % and 40% for swell water and to 17% or more for the washing steps. The total amount of water used could be decreased with at least 20 %. The water used came out as both liquid and solid fraction. The concentration of analyzed compounds were very low (<0.1%) in the liquid part. The

solid part however, had 60 % of starch and 40 % of unknown compounds. Half of the minerals and vitamins were lost from the bran. Future studies need to focus on the solid part from the de-starching process of wheat bran since it potentially consists of arabinoxylans, proteins and phytochemicals. This project contributes with data that could be a part of decreasing the water use during de-starching. It also narrows down where the potentials of wheat bran fractionation could be. Knowledge that is in line with the Global Goal For Sustainable Development of the United Nations.